DISSERTATION

on

A STUDY ON THE COAGULATION PROFILE AND ITS CLINICOPATHOLOGICAL CORRELATION OF THE HAEMOPHILIA PATIENTS AT THE DAY CARE CENTRE OF TIRUNELVELI MEDICAL COLLEGE

submitted in partial fulfillment of the requirements for the degree of

Doctor of Medicine(BRANCH-III)

M.D. PATHOLOGY

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY CHENNAI

TIRUNELVELI MEDICAL COLLEGE
TIRUNELVELI
MAY 2019

CERTIFICATE

This is to certify that the dissertation titled "**A STUDY ON THE COAGULATION PROFILE AND ITS CLINICOPATHOLOGICAL CORRELATION OF THE HAEMOPHILIA PATIENTS AT THE DAY CARE CENTRE OF TIRUNELVELI MEDICAL COLLEGE",** is a bonafide work done by **Dr.S.VISHNU PRIYA**, Post Graduate Student, Department of Pathology, Tirunelveli Medical College,Tirunelveli – 627011, in partial fulfilment of the university rules and regulations for the award of MD DEGREE in PATHOLOGY BRANCH-III, under my guidance and supervision, during the academic period from 2016to 2019.

Prof.Dr.S.M.KANNAN, MS.,M.Ch.,
Dean,
Tirunelveli Medical College,
Tirunelveli- 627011.

CERTIFICATE

I hereby certify that this dissertation entitled "**A STUDY ON THE COAGULATION PROFILE AND ITS CLINICOPATHOLOGICAL CORRELATION OF THE HAEMOPHILIA PATIENTS AT THE DAY CARE CENTRE OF TIRUNELVELI MEDICAL COLLEGE",** is a record of work done by **Dr.S.VISHNU PRIYA**, in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during her postgraduate degree course period from 2016- 2019. This work has not formed the basis for previous award of any degree.

Prof. Dr.ARASI RAJESH. MD.,
Department of pathology,
Tirunelveli Medical College,
Tirunelveli- 627011.

Prof.Dr.K. SHANTARAMAN.MD.,
Professor and Head,
Department of Pathology,
Tirunelveli Medical College
Tirunelveli- 627011.

DECLARATION

I solemnly declare that the dissertation titled "**A STUDY ON THE COAGULATION PROFILE AND ITS CLINICOPATHOLOGICAL CORRELATION OF THE HAEMOPHILIA PATIENTS AT THE DAY CARE CENTRE OF TIRUNELVELI MEDICAL COLLEGE",** was done by me at Tirunelveli Medical College, Tirunelveli– 627011, during the period March 2017 to September 2018 under the guidance and supervision of **Prof.Dr.ARASI RAJESH, MD.**, to be submitted to The Tamil Nadu Dr. M.G.R. Medical University towards the partial fulfilment of requirements for the award of MD DEGREE in PATHOLOGY BRANCH-III.

Place : Tirunelveli

Date :

Dr. **S.VISHNU PRIYA**,
Post Graduate Student,
Department of Pathology
Tirunelveli Medical College,
Tirunelveli – 627011.

TIRUNELVELI MEDICAL COLLEGE

INSTITUTIONAL RESEARCH ETHICS COMMITTEE
TIRUNELVELI, STATE OF TAMILNADU, SOUTH INDIA PIN 627011
91-462-2572733-EXT; 91-462-2572944; 91-462-2579785; 91-462-2572611-16
online@tvmc.ac.in, tirec@tvmc.ac.in; www.tvmc.ac.in

CERTIFICATE OF REGISTRATION & APPROVAL OF THE TIREC REF NO:998/PATHO/2017

PROTOCOL TITLE: A STUDY ON THE COAGULATION PROFILE AND ITS CLINICOPATHOLOGICAL CORRELATION OF THE HAEMOPHILIA PATIENTS AT THE DAY CARE CENTRE OF TIRUNELVELI MEDICAL COLLEGE
PRINCIPAL INVESTIGATOR: Dr.S.VISHNU PRIYA, MBBS.,
DESIGNATION OF PRINCIPAL INVESTIGATOR:
DEPARTMENT & INSTITUTION: TIRUNELVELI MEDICAL COLLEGE, TIRUNELVELI

Dear, Dr.S.VISHNU PRIYA, MBBS., The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 10.03.2017

THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

1. TIREC Application Form ☐
2. Study Protocol ☐
3. Department Research Committee Approval ☐
4. Patient Information Document and Consent Form in English and Vernacular Language ☐
5. Investigator's Brochure ☐
6. Proposed Methods for Patient Accrual Proposed ☐
7. Curriculum Vitae of the Principal Investigator ☐
8. Insurance /Compensation Policy ☐
9. Investigator's Agreement with Sponsor ☐
10. Investigator's Undertaking ☐
11. DCGI/DGFT approval ☐
12. Clinical Trial Agreement (CTA) ☐
13. Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA) ☐
14. Clinical Trials Registry-India (CTRI) Registration ☐

THE PROTOCOL IS APPROVED IN ITS PRESENTED FORM ON THE FOLLOWING CONDITIONS

1. The approval is valid for a period of 2 year/s or duration of project whichever is later
2. The date of commencement of study should be informed
3. A written request should be submitted 3weeks before for renewal / extension of the validity
4. An annual status report should be submitted.
5. The TIREC will monitor the study
6. At the time of PI's retirement/leaving the institute, the study responsibility should be transferred to a person cleared by HOD
7. The PI should report to TIREC within 7 days of the occurrence of the SAE. If the SAE is Death, the Bioethics Cell should receive the SAE reporting form within 24 hours of the occurrence.
8. In the events of any protocol amendments, TIREC must be informed and the amendments should be highlighted in clear terms as follows:
   a) The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
   b) The PI must comment how proposed amendment will affect the ongoing trial. Alteration in the budgetary status, staff requirement should be clearly indicated and the revised budget form should be submitted.
   c) If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented.
   d) If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IEC, only then can they be implemented.
   e) Approval for amendment changes must be obtained prior to implementation of changes.
   f) The amendment is unlikely to be approved by the IEC unless all the above information is provided.
   g) Any deviation/violation/waiver in the protocol must be informed.

STANDS APPROVED UNDER SEAL

Dr.K.Shantaraman MD
Registrar, TIREC
Tirunelveli Medical College, Tirunelveli – 627011
State of Tamilnadu, South India

Dr.J.Suresh Durai, MD
Member Secretary, TIREC
Tirunelveli Medical College, Tirunelveli – 627011
State of Tamilnadu, South India

ACKNOWLEDGEMENT

I thank **Professor Dr.S.M.KANNAN, M.S**,**M.Ch**., Dean, Tirunelveli Medical College, for having permitted me to conduct the study and use the hospital resources in the study.

I express my heartfelt gratitude to **Professor Dr. SHANTARAMAN. K. MD**, Professor and Head, Department of Pathology, for his inspiration, advice and guidance in making this work complete.

I am extremely thankful to **Professors Dr.SWAMINATHAN .K. M.D., Dr.SURESH DURAI. J. M.D., Dr.ARASI RAJESH, M.D., Dr.VASUKI M.D.** I am thankful to **Dr.V.BHAGIYALAKSHMI, M.D.,** Associate Professor, Department of Pathology, for guiding me during the period of study.

I am extremely thankful to Assistant Professors**,** Dr.Johnsy Merla M.D., Dr.Hidhaya Fathima M.D., Dr.Sindhuja M.D., Dr.Mahalakshmi M.D., Dr.Dina Mary MD., Dr. Dharma saranya M.D., Dr.Chandhru Mari MD., Dr.Selin sofia MD., Dr.Gurusamy MD, Dr.Ramasundari Ilambirai MD., Department of Pathology, for guiding me academically and professionally during the period of study.

I thank **Professor Dr.Arumugapandian S Mohan M.D., Professor**

Dr. Ravichandran Head of the department, Department of medicine, for having permitted me to conduct the study in their department premises.

I also thank all the lab technicians and my fellow postgraduates for their cooperation which enormously helped me in the study. Without their humble cooperation, this study would not have been possible.

I thank **GOD AND MY PARENTS**, for blessing me not only in this study, but in all endeavours of my life.

CERTIFICATE – II

This is to certify that this dissertation work title **"A STUDY ON THE COAGULATION PROFILE AND ITS CLINICOPATHOLOGICAL CORRELATION OF THE HAEMOPHILIA PATIENTS AT THE DAY CARE CENTRE OF TIRUNELVELI MEDICAL COLLEGE"** of the candidate **Dr.S.VISHNU PRIYA** with registration Number **201613307** for the award of **M.D.** Degree in the branch of **PATHOLOGY (III).** I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion page and result shows **2 percentage** of plagiarism in the dissertation.

Guide & Supervisor sign with Seal.

URKUND

Urkund Analysis Result

Analysed Document:	A STUDY ON THE COAGULATION PROFILE AND ITS CLINICOPATHOLOGICAL CORRELATION OF THE HAEMOPHILIA PATIENTS AT THE DAY CARE CENTRE OF TIRUNELVELI MEDICAL COLLEGE.pdf (D42271380)
Submitted:	10/8/2018 1:10:00 PM
Submitted By:	svishnupriya31@gmail.com
Significance:	2 %

Sources included in the report:

HEMOPHILIC ARTHROPATHY-CLINICAL,FUNCTIONAL AND RADIOLOGICAL EVALUATION.docx (D31188562)
pliagarism.docx (D42105034)
pliagarism.docx (D42141521)
https://www.duo.uio.no/handle/10852/36079
https://www.guidelinecentral.com/summaries/guidelines-for-the-management-of-hemophilia/
https://en.wikipedia.org/wiki/Hemophelia
http://www.bloodjournal.org/content/113/1/11?sso-checked=true

Instances where selected sources appear:

12

CONTENTS

1.	**INTRODUCTION**	1
2.	**AIMS OF THE STUDY**	2
3.	**REVIEW OF LITERATURE**	3
4.	**MATERIALS AND METHODS**	60
5.	**OBSERVATIONS AND RESULTS**	64
6.	**DISCUSSION**	89
7.	**SUMMARY AND CONCLUSION**	99
8.	**BIBILIOGRAPHY**	
9.	**ANNEXURES**	
	ANNEXURE I – MASTER CHART	
	ANNEXURE II – PROFORMA	
	ANNEXURE III- CONSENT FORM	

ABBREVIATIONS

ADAMTS-13 -A disintegrin and metalloproteinase with thrombospondin motifs 13

APC - Activated protein C
APTT - Activated Partial Thromboplastin Time
C4BP - C4b–binding protein
CBC – Complete Blood Count.
CT - Computed Tomography
CVC - Central Venous Catheter
CXCL7 – Chemokine (C-X-C) Ligand 7
DDAVP - Desmopressin
EACA - ε-aminocaproic acid
F VIIIa- Activated Factor VIII
FFP - Fresh frozen plasma
FISH - Functional Independence Score in Hemophilia
FIX - Factor IX
FVa- Activated Factor V
FVIIa – Activated Factor VII
FVIII - Factor VIII
FXa- Activated Factor X
FXI - Factor XI
FXIa - Activated Factor XI
FXII - Factor XII
FXIIa - Activated Factor FXII
FXIIIa - Activated Factor FXIII
GPIb – Glycoprotein Ib
HIV - Human Immunodeficiency Virus
HK - High-molecular-weight kininogen
ICH - Intracranial Hemorrhages
IL-1 – Interleukin 1
IL-6 – Interleukin 6
INR - International normalized ratio
MRI - Magnetic Resonance Imaging
NSAIDs – Non Steroidal Anti Inflammatory Drugs
PF4 – Platelet Factor 4

PK – Prekallikrein
PNP - Pooled normal plasma
PROS1 - Protein S
PT - Prothrombin Time
RANTES – Regulated On Activation Normal T-cell Expressed and Secreted
rFVIII - Recombinant FVIII
ROM – Range Of Movements
TAFI - Thrombin-activatable fibrinolysis inhibitor
TAFI a - Activated TAFI
TF – Tissue Factor
USG - Ultra-sonography
VWF – Von Willebrand factor
WFH - World Federation of Haemophilia

INTRODUCTION

Hemophilia is the oldest recognized inherited bleeding disorder[1]. It is a disorder of coagulation system that occurs due to deficiency of clotting factors which takes part in coagulation cascade. India has the second highest burden of haemophilia patients in the world[2]. According to the World Federation of Haemophilia (WFH) the total number of Haemophilia patients universally is about 4,00,000[3]. The prevalence of hemophilia is estimated to be about 1:10,000 birth[4][6]. People with Haemophilia bleedcontinuously as blood is unable to clot because of deficiency of clotting factors[5]. Bleeding can occur into joints like knees, ankles, elbow. Bleeding into internal organs like brain is a serious complication in patients with haemophilia . Bleeding may be caused by an injury or spontaneous bleeding can occur in severe form of the disease[5]. The two common forms of haemophilia are hemophilia A and hemophilia B. They arise as a consequence of having insufficient amounts of coagulation factor VIII (FVIII) and factor IX (FIX) respectively. Both hemophilia A and B are X-linked recessive disorders[7]. So the disorders manifest almost entirely in males and females are the carriers of the mutated gene only[5].

AIMS AND OBJECTIVES:

The present study was aimed at analyzing the Haemophilia patients admitted to the Day Care Centre of Tirunelveli Medical College Hospital during the period between March 2017 and September 2018 with the following objectives.

OBJECTIVES:

I. Comparative analysis of the coagulation profile, severity of the disease, and physical disability.
II. Evaluation of relevant laboratory parameters – CBC, Coagulation profile.
III. Evaluation of long term complications of factor concentrates therapy.

REVIEW OF LITERATURE

I . HISTORY OF HAEMOPHILIA:

The word Haemophilia is derived from the Greek word "*haima*" which means blood and "*philia*" which means friend[8]. In Arabic language; haemophilia means Naaor meaning the unstopped bleeding vessel.

Haemophilia in the Ancient period:

The study of blood coagulation can be traced back to 400 BC when the father of medicine, Hippocrates observed that the blood of a wounded soldier consealed as it cooled. He also noticed that bleeding from a small wound stopped as skin covered the blood. If the skin was removed, bleeding started again[9]. X-linked Haemophilia was a disease known to the ancient world with the earliest references available. Around second century AD. Rabbinical rulings exempted male boys from circumcision if two previous brothers had died of bleeding after the procedure[10]. Rabbi Simon ben Gamaliel stopped a boy to be circumcised as the sons of his mother's three elder sisters had died after circumcision.

Al-Zahrawi - Albucasis (936-1013 AD), the famous physician of the Islamic empire in his medical book "*Kitab al-Tasrif",* described a disease which he named as blood disease[8]. His description about the blood disease corresponds with haemophilia.

Haemophilia in the Recent Age:

Recent descriptions of haemophilia are there from the end of the 18th century. In 1803, Dr. John Conrad Otto (1774-1844), an American physician, published an account about " A hemorrhagic disposition existing in certain families" in the "New York Medical Repository"[11]. He found out that the disorder was hereditary and although it affected only males the disorder was transmitted by unaffected females to a proportion of their sons.

The name 'haemophilia' meaning 'love of blood' appeared in the title of Hopff's treatise of 1828 published at the University of Zurich. Involvement of joints, which is the most characteristic symptom of haemophilia, was described in detail by Konig in 1890.

Haemophilia "The Royal disease" :

Haemophilia was figured prominently in the history of European royalty in the 19th and 20th centuries. Queen Victoria, through two of her five daughters (Princess Alice and Princess Beatrice), passed the mutation to various royal houses across the continent, including the royal families of Spain, Germany and Russia. Victoria's son Leopold suffered from the disease. For this reason, haemophilia was popularly called as "the royal disease"[12]. The spread of hemophilia in the royal families of Europe was a very important factor in the development of medical knowledge about the disease.

Haemophilia in the Last Century:

Paul Oskar Morawitz (1879-1936) in 1905 assembled the coagulation factors into the scheme of coagulation and he also demonstrated it in the presence of Queen Victoria and her family[8].

Initially four-factor concept of clotting was discovered. It proposed that interaction between calcium (Factor IV) ,tissue thromboplastin (Factor III)and prothrombin (Factor II) converts fibrinogen (Factor I) into a fibrin clot. Paul Owren, in 1944, first discovered a bleeding patient who defied the four-factor concept of clotting. Owren observed that a cofactor was involved in the conversion of prothrombin to thrombin and this lead to the discovery of factor V. Factor VIII was discovered in 1937 by American researchers A.J. Patek and F.H.L. Taylor[13]. They found that intravenous administration of plasma precipitates leads to shortening blood clotting time. It was later called by Taylor as anti-hemophilic globulin (Factor VIII). In 1939, American pathologist Kenneth Brinkhous showed that people with hemophilia have a deficiency in the plasma factor he later called anti-hemophilic factor (Factor VIII).

In 1952, Christmas factor or factor IX was discovered which was named after the first patient, Stephen Christmas who suffered the disease[14]. In 1957, Factor X deficiency was described in a woman named Prower and a man named Stuart[15]. Factor XI deficiency was described in 1953 as a milder bleeding tendency[16]. In 1955 factor XII or Hageman factor deficiency was identified in

a patient who died from a thrombotic stroke[17]. In 1960, Duckert described patients who had a bleeding disorder and characteristic delayed wound healing. This fibrin stabilizing factor was called factor XIII[18].

II. Etiology and Types of Haemophilia

Haemophilia is an X-linked recessive disorder caused due to deficiency or complete absence of some clotting factor. Types of haemophilia includes

1. Haemophilia A
2. Haemophilia B
3. Haemophilia C
4. Haemophilia B Leyden
5. Acquired haemophilia

Haemophilia A:

It is called as Classic Haemophilia[5]. Incidence of Haemophilia A is 1:5000-10,000 births[19].It is caused by deficient plasma concentration of clotting factor VIII. It occurs as an X-linked recessive disorder affecting only males. Females are carriers of this disease. Factor VIII is an essential protein for blood coagulation which circulates in an inactive form bound to Von Willebrand factor and protects factorVIII from proteolytic degradation[20]. Carrier females are usually asymptomatic but can have bleeding symptoms like easy bruises, menorrhagia or excess bleeding after trauma. Bleeding symptoms

are due to significant reduction in F VIII levels caused by greater inactivation of normal F VIII gene.

Haemophilia B:

It is also called as Christmas disease and occurs due to deficiency of the clotting factor IX. Incidence of haemophilia B is 1: 20,000-34,000[5]. It was initially named "christmas disease" after the first person Stephen Christmas who was diagnosed with the disorder in 1952[21].This is inherited in an X-linked recessive pattern similar to haemophilia A.

Haemophilia C:

It is caused by absence or deficiency of Factor XI. Incidence of Haemophilia C is 1: 1,00,000[5]. It is inherited in an autosomal recessive pattern. So, both the parents must carry the defective gene for the disease to be manifested in the offspring. This disease affects both males and females in equal numbers. There are few exceptions where people may have bleeding problems when only one of their parents has the defective gene for Factor IX deficiency.

Haemophilia B Leyden:

It is an unusual form of factor IX deficiency[22]. Haemophilia B leyden is named after a place in Netherlands where it was first described. Classic haemophilia B is a life long disorder whereas individuals with haemophilia B

leyden usually outgrow the disorder at puberty or adulthood. Incidence of this disease is around 3%[23]. It occurs due to single point mutation in the promoter region of factor IX gene[24]. These patients have low plasma levels(≤ 1 to 13%of normal) of blood coagulation factor IX during adulthood. This factor IX level gradually increases during adulthood. By midlife their factor IX level increases and reach a low end of normal range. This occurs under the influence of androgens[22]. These patients later no longer require treatment for their bleeding episodes.

Acquired haemophilia:

It is a type of autoimmune disorder. It occurs as a severe bleeding disorder affecting both males and females. It is due to production of autoantibodies in adult life which causes inactivation of factor VIII[25].

III. Classification of haemophilia

Haemophilia is classified as

a) Mild Haemophilia:

Patients with mild haemophilia have 6-40%(0.06-0.400/ml) of normal F VIII activity[7] . Haemorrhage secondary to trauma or surgery occurs in these patients; Rare spontaneous haemorrhage can also occur.

b) Moderate Haemophilia:

Patients with moderate haemophilia have 1-5% (0.01-0.050/ml)of normal F VIII activity[7] . Haemorrhage secondary to trauma or surgery occurs in these patients; Occasional spontaneous hemarthroses can also occur.

c)Severe Haemophilia:

Patients with severe haemophilia have ≤1%(<0.01 u/ml)of normalF VIII activity[7]. These patients have spontaneous haemorrhage from early infancy, frequent spontaneous hemarthroses and other haemorrhages. It requires clotting factor replacement therapy as treatment.

The severity of the disease is determined by the residual clotting factor activity.

HAEMOSTASIS AND BLOOD COAGULATION

Blood coagulation is a part of important host defense mechanism termed hemostasis. Blood is a liquid that circulates through the vasculature under pressure. On injury to the vessels , the escaping blood must rapidly be converted into a "clot" to plug the hole and minimize further blood loss[26]. The blood plasma contains a number of soluble proteins called as coagulation factors which act together in a cascade of enzyme activation events, resulting in the formation of a fibrin clot[20]. Hemostasis is the physiologic process by which the clotting cascade of events seals up the vascular damage to limit blood

loss following injury. On vessel injury, platelets adhere to macromolecules (i.e. collagen) present in the subendothelial tissues and aggregate to form a platelet plug (primary hemostasis)[28]. Activated platelets leads to local activation of plasma coagulation factors which initiates a sequential amplifying cascade, resulting in the formation of a cross-linked fibrin clot that further strengthens the platelet plug (secondary hemostasis)[29].

Thrombosis includes a group of pathologic conditions in which the clotting cascade is triggered inside the lumen of a blood vessel, leading to the formation of a blood clot called as a "thrombus" which impedes the blood within a vessel.

SEQUENTIAL STEPS IN BLOOD CLOTTING

1) Vasoconstriction[30]

   Vasospasm at the site of injury , limits the blood flow to that area.

2) Platelet activation[30]

   Platelets are activated by Thrombin and aggregate at the site of injury forming a temporary, loose platelet plug.

3) Formation of strong Fibrin clot[30].

Blood coagulation is the process of interaction between large number of plasma glycoproteins with blood platelets and vascular endothelial cells.

The Three pathways of Blood clotting which leads to fibrin production are:

1) Tissue Factor Pathway (Extrinsic)
2) Contact Activation pathway (intrinsic)
3) Common pathway.

The Extrinsic or Tissue Factor Pathway

The plasma clotting cascade i.e., coagulation cascade is a series of reactions involving the zymogen (inert precursors of enzymes) activation by means of limited proteolysis. The protein cofactors involved in the clotting cascade generally circulate in the plasma as inert procofactors .These procofactors must be converted into active cofactors by limited proteolysis. The Blood clotting proteins are denoted by Roman numerals. To represent the active form of the clotting factor a lower case "a" is added to the numeral after proteolytic activation[31].

There are 13 clotting factors . They are

1. Factor I – Fibrinogen
2. Factor II – Prothrombin
3. Factor III – Tissue thromboplastin
4. Factor IV – ionized calcium (ca++)
5. Factor V – Labile factor or proacclerin
6. Factor VI – unassigned
7. Factor VII – stable factor or proconvertin
8. Factor VIII – antihaemophilic factor

9. Factor IX – Plasma thromboplastin component, Christmas factor
10. Factor X – Stuart –Prower factor
11. Factor XI – Plasma thromboplastin antecedent
12. Factor XII – Hageman factor
13. Factor XIII – Fibrin – stabilizing factor

The process of blood coagulation can be studied under three phases. They are

- Initiation phase;
- Phase of amplification and
- Phase of propagation.

Initiation phase :

During the initiation phase membrane-bound tissue factor (TF) first interacts with FVII and converts it to FVIIa which catalyzes the activation of both FIX and FX. FX is the most efficient substrate. Activated FX i.e FXa binds to the platelet membrane and it cleaves prothrombin to thrombin. The small amount of thrombin which is initially formed activates a number of factors that are critical for phase of propagation; namely factors V, VIII and XI. Other important role is the activation of platelets which provide the surface on which the propagation phase of coagulation occurs. Phosphatidylserine exposed on the activated platelets provides binding sites for procoagulant zymogens and enzymes like factors IXa, VIIIa, X, Xa,Va and II.[33]

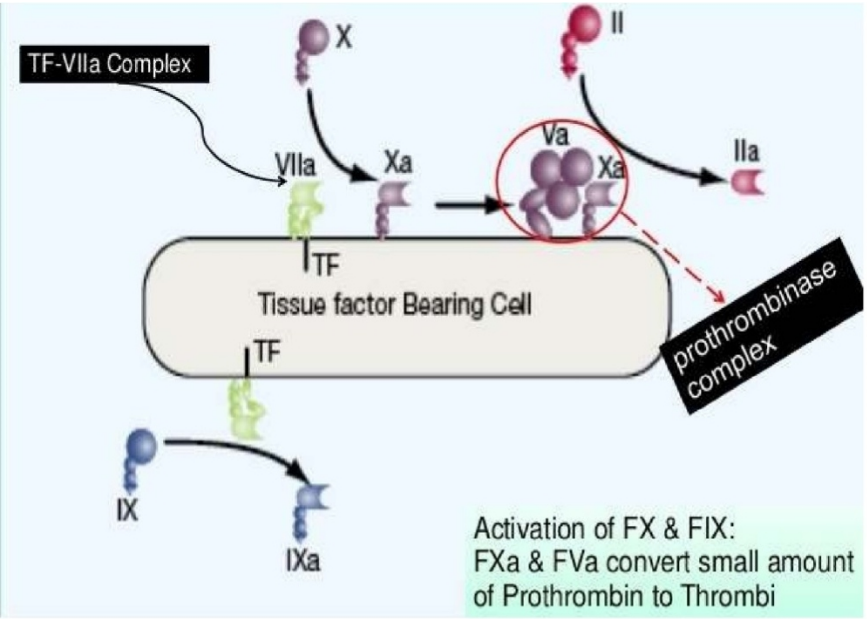


Fig:1.Initiation phase of coagulation.

Adapted from: Biswaprakash Patri, Anubhav Abinash Sahu The Cell Based Model of in-vivo Coagulation:A Work in ProgressDOI: 10.9734/IBRR/2016/26906

Phase of amplification

Factor VIIa / TF activated factor Xa promotes small scale thrombin generation. FXa activates other cofactors like factor VIII,V and platelets . FXa complexes with FVa to activate small amounts of prothrombin to thrombin[33].Thrombin then activates FVIII to FVIIIa which interacts with FIXa to play a key role in coagulation.

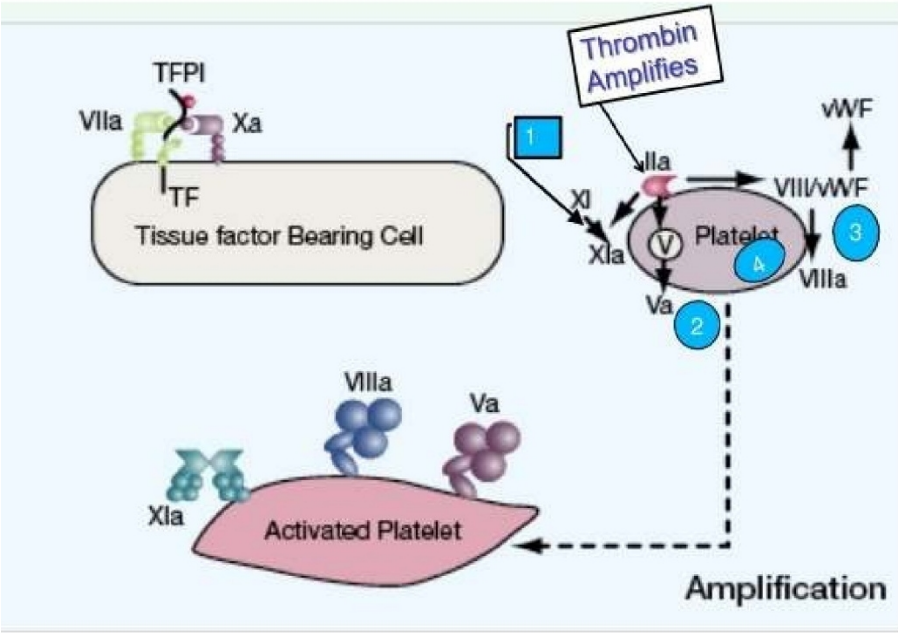


Fig :2Amplification phase of coagulation.

Adapted from: Biswaprakash Patri, Anubhav Abinash Sahu The Cell Based Model of in-vivo Coagulation:A Work in ProgressDOI: 10.9734/IBRR/2016/26906

Phase of propagation

In the propagation phase,the intrinsic activites are supported by the activated platelet surface,that produces a large scale thrombin burst[33]. In the intrinsic tenase complex, membrane-bound FIXa forms a complex with its cofactor FVIIIa andcalcium. This complex is the major activator of FX and is 50 times more active than the FVIIa–TF complex. More than 90% of the FXa produced in the coagulation cascade is produced by the intrinsic tenase complex. The F Xa produced by F IXa – F VIIIa is essential for the generation of thrombin burst during the propagation phase of coagulation. This produces >95% of circulating thrombin. Thrombin which is formed helps in conversion of fibrinogen to fibrin.

Thrombin also causes activation of FXIII to FXIIIa which catalyzes the formation of covalent bonds. This cross linking leads to increase in the elasticity of individual fibres in the clot[34].

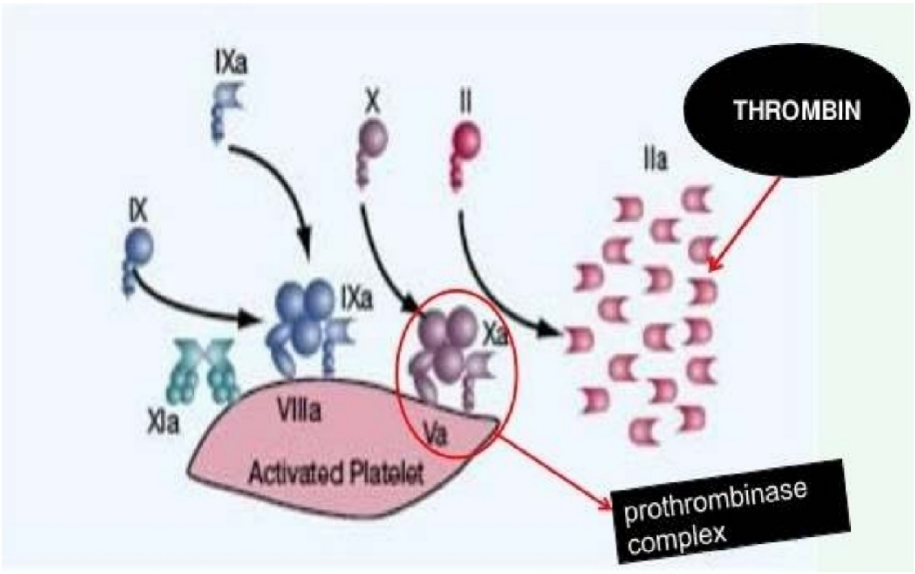


Fig :3Propagation phase of coagulation.

Adapted from: Biswaprakash Patri, Anubhav Abinash Sahu The Cell Based Model of in-vivo Coagulation:A Work in ProgressDOI: 10.9734/IBRR/2016/26906

When Haemostatic functions fail, haemorrhage or thromboembolic phenomenon results.

The contact Activation pathway or the Intrinsic Pathway:

The contact pathway of initiating blood clotting is also termed as the “intrinsic” pathway. Intrinsic pathway can be triggered without addition of TF to the blood or plasma. This pathway is triggered when plasma comes into contact with some artificial surfaces like Glass test tubes, celite and clay etc. The contact

pathway does not contribute to normal hemostasis, it is thought to participate in thrombotic diseases.

The contact pathway of coagulation is initiated by activation of factor XII in a process that also involves high-molecular-weight kininogen and plasma prekallikrein[31] . Contact of blood with any artificial surface leads to a change in the conformation of FXII, leading to the formation of small amounts of active factor XII [35]. This enzyme i.e., active FXII (FXIIa) activates plasma PK to kallikrein. Further reciprocal activation of FXII by kallikrein, and PK by FXIIa, results in a positive feedback loop .The FXIIa that is produced further activates the downstream substrate, FXI, to FXIa . FXIa causes limited proteolysis of FIX to FIXa this leads to the formation of "intrinsic tenase" complex (i.e., the cell-surface complex of FIXa and FVIIIa)[36]. The intrinsic tenase complex causes activation of FX to FXa. The final common pathway of blood clotting further leads to thrombin generation and formation a blood clot. The contact pathway plays an important role in clot formation in vitro, and it has no contribution to hemostasis in vivo . The Components of the contact system also contribute to fibrinolysis, and inhibit thrombin-induced platelet activation, angiogenesis and other adhesive interactions[37].

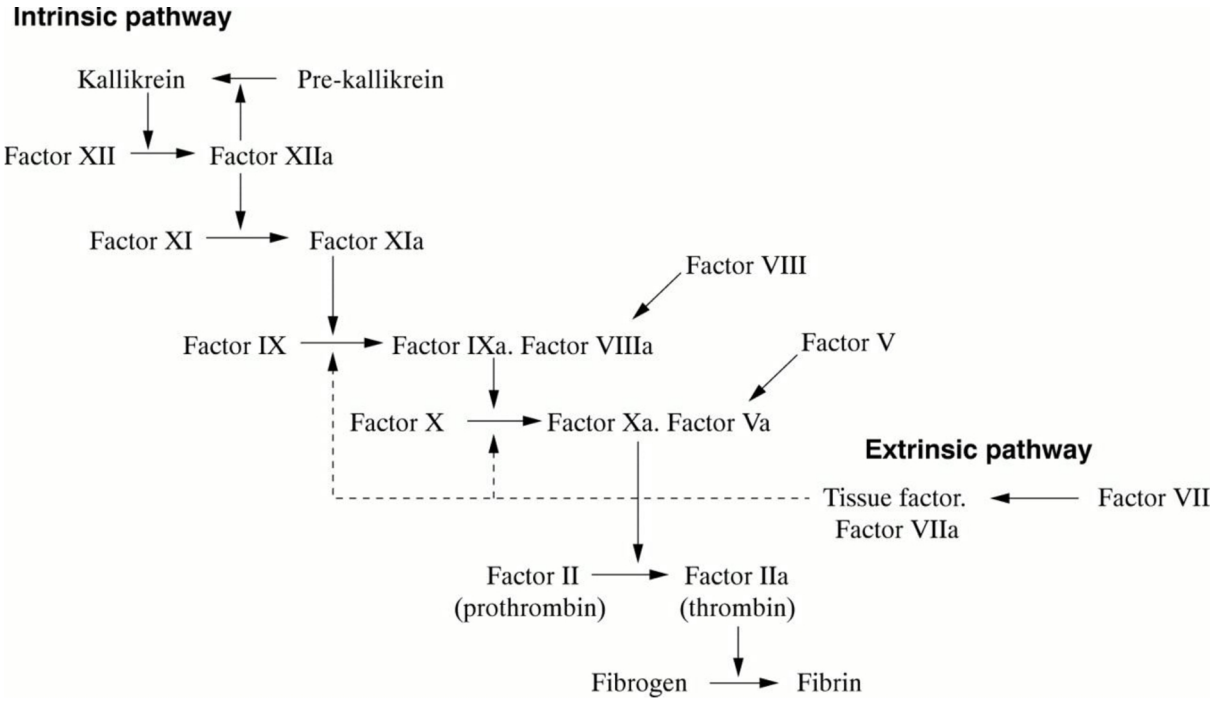


Fig :4 Intrinsic and Extrinsic system of coagulation cascade.

Adapted from: World Journal Of Pharmacy And Pharmaceutical Sciences Hemophilia-Inherited Bleeding Disorder: An Overview 07 March 2014

CLOTTING FACTORS

FIBRINOGEN(FACTOR I)

Fibrin derived from fibrinogen is the structural meshwork that forms the initial platelet plug which is then converted into a solid hemostatic clot. Fibrinogen is synthesized in the liver and has a plasma half-life of 3 to 5 days[38]. Fibrinogen is a dimeric glycoprotein. Thrombin binds to the central domain of fibrinogen and proteolytically releases two fibrinopeptides A and two fibrinopeptides B from each fibrinogen molecule[39]. Polymerization of fibrin monomers occur during which other plasma proteins like fibronectin, thrombospondin, and

VWF also bind to the surface of the developing meshwork. These surface proteins helps in the generation, crosslinking, and lysis of fibrin. Thrombin activates factor XIII, which stabilizes the fibrin polymer by crosslinking. The fibrin mesh which is formed can be degraded by the fibrinolytic system. Plasmin cleaves fibrin and fibrinogen into soluble degradation products called D-dimer[40]. D-dimer assay is used as a marker of activated coagulation. In addition to its procoagulant role in stabilizing the initial platelet hemostatic plug, it also act as an important inhibitor of thrombin generation. The genes for fibrinogen is located on chromosome 4q23-q32[20]. Afibrinogenemia and dysfibrinogenemias are associated with bleeding diathesis.

PROTHROMBIN (FACTOR II)

It is a vitamin K-dependent zymogen. Plasma prothrombin is primarily synthesized in the liver. It has a plasma half-life of about 60 hours[20]. Prothrombin is proteolytically activated by the prothrombinase complex (i.e., factor Va, factor Xa, calcium, and anionic phospholipids) to generate procoagulant α-thrombin (IIα) . Thrombin is the initiator of procoagulant pathways by the proteolytic activation of cofactors V and VIII and zymogen factor XI which collectively amplify thrombin and fibrin formation. It also causes activation of factor XIII that crosslinks and stabilizes the fibrin polymers. Another procoagulant function of thrombin is to inhibit fibrinolysis by proteolytic activation of the thrombin-activatable fibrinolysis inhibitor

(TAFI). In addition to its procoagulant activity, thrombin has an anticoagulant function. Thrombin binds to the cofactor thrombomodulin on endothelial cells, activates protein C which causes inactivation of factors Va and VIIIa. The gene for prothrombin is located on chromosome 11p11.2[41] . When there is homozygous or compound heterozygous loss of function mutations in the prothrombin gene it leads to a bleeding tendency. If there is gain-of-function mutations in the prothrombin gene it is associated with increased risk of thrombosis.

TISSUE FACTOR

Tissue factor or thromboplastin is the cellularreceptor for factors VII and VIIa. It acts as a cofactor for coagulation factors.It is expressed in fibroblasts , smooth muscle cells and is not exposed to blood. The tissue factor–factor VIIa complex plays a major role in the initiation of blood coagulation.An injury allows the extravascular tissue factor to form a complex with factor VIIa in blood and initiate blood coagulation.

FACTOR V

Factor V is primarily synthesized in the liver and it circulates in the plasma as a large single-chain procofactor consisting of 2196 amino acids with a half-life of 12 to 36 hours[20] . Factor V is also called as proacclerin. Around 20 percent of the total factor V in blood is stored in the platelets α-granules .On activation of platelet, platelet factor V is available at the site of injury. The local concentrations of platelet factor V will exceed the factor V plasma

concentration by more than 100 fold[42].FactorV undergoes extensive post translational modifications by means of sulfation, phosphorylation, and *N*-linked glycosylation. Sequential proteolytic cleavage of the procofactor factor V leads to the synthesis of cofactor Va. Factor Va acts as a non enzymatic cofactor within the prothrombinase complex, this increases the ability of factor Xa to rapidly convert prothrombin to thrombin. The gene for factor V is located on chromosome 1q23. Loss-of-function mutations in the factor V gene lead to a bleeding disorder which is termed as parahemophilia or Owren parahemophilia[43].

FACTOR VII

Factor VII is synthesized in the liver and it circulates in the plasma as a single-chain zymogen containing 406 aminoacids with a short plasma half-life of 3 to 6 hours[20]. Factor VII is proteolytically activated on forming a high-affinity complex with its cofactor tissue factor. The gene encoding factor VII is located on chromosome 13q34. Factor VII deficiency is one of the inherited rare bleeding disorders.

FACTOR VIII:

F VIII is produced primarily by the liver. Hepatic endothelial cells are the major factor producing cells. It is also secreted by spleen, lymph nodes and kidney as an inactive single chain protein. It acts as a cofactor for factor IX a; this cofactor in the presence of Ca++ and phospholipids forms a complex which

converts F X to activated form X a which is an important step in the coagulation cascade[66]. This factor F VIII circulates in a non covalent complex with VWF. Half life of F VIII associated with VWF is 8-12 hours; it is markedly reduced in the absence of VWF. The functions of vWF are to stabilize FVIII and mediate platelet adhesion and aggregation at the site of injury. Free plasma FVIII not bound to vWF is rapidly inactivated by activated protein C (which complexes with cofactor protein S) and cleared by low-density lipoprotein-related protein. When bound to vWF, FVIII is resistant to all circulating proteolytic enzymes except thrombin[67]. F VIII is activated by thrombin or F X a resulting in formation of F VIII a. Activation also releases VWF from F VIII. F VIII has 6 tyrosine residues and are modified by sulfation. Sulfation is essential for activation of thrombin; maximal activity on complex formation with factors s IX a and maximal affinity of factor VIII a for VWF [69]. Thrombin activation of FVIII coincides with the cleavage of its heavy and light chains, causing it to dissociate from vWF to act as a cofactor to FIXa. In the presence of calcium and a negatively charged phospholipid surface on activated platelets and other cells, the FIXa-FVIIIa complex catalyzes the activation of FX to a greater efficiency than the FVIIa-TF complex.

FACTOR IX

Factor IX is known as "Christmas factor," named after one of the first identified hemophilia B patients[44]. Synthesis of Factor IX occurs in the liver

and it circulates in plasma as a single-chain zymogen containing 415 amino acids with a half-life of 18 to 24 hours. Proteolysis of factor IX by either the tissue factor–factor VIIa complex or factor XIa results in the generation of factor IXa. The gene encoding factor IX is located on chromosome Xq27.1[45]. A defect or deficiency in factor IX leads to hemophilia B.

FACTOR X

Factor X is known as the "Stuart-Prower factor," named after the first two patients who had deficiency of factor X[46]. Primary synthesis of Factor X takes place in the liver and it circulates in plasma as a two-chain zymogen containing 445 amino acids . It has a half-life of 34 to 40 hours. Factor X is proteolytically activated by either the factor VIIIa–factor IX("intrinsic tenase") or the tissue factor–factor VIIa ("extrinsic tenase")enzyme complexes .This leads to the release of the activation peptide and generation of factor Xa. The gene encoding factor X is located on chromosome 13q34 and it spans almost 27 kb with 8 exons[47]**.** Factor X deficiency leads to the most severe of the rare congenital bleeding disorders

FACTOR XI

Factor XI is synthesized in the liver and it is secreted as a single-chain zymogen of 607 amino acids. The plasma half-life of Factor XI is around 60 to 80 hours.In the circulation factor XI homodimers complex with high-molecular-weight kininogen (HK)[48].Activation of a factor XI subunit to factor XIa occurs by proteolysis of N-terminal region of the serine protease domain to yield two-

chain activated factor XIa. The catalysts which are capable of factor XI activation are the contact factor XIIa,thrombin, or factor XIa itself due to the presence of negatively charged surfaces. The human factor XI gene is located on the chromosome 4q35 . Deficiencies of factor XI in human beings leads to a bleeding tendency but it is not as severe as in hemophilia A or B. Ashkenazi Jews in Israel are prone for factor XI deficiency. Increased levels of factor XI are associated with a risk of venous thrombosis[49].

FACTOR XII

Factor XII was discovered around 1955 as the "Hageman factor,"[50]. It is synthesized in the liver and circulates in plasma as a single-chain zymogen of 596 amino acids. It has a half-life of 50 to 70 hours. Factor XII is converted to*α*-factor XIIa on contact with a negatively charged surface. The negatively charged surfaceinduces a conformational change in factor XII[51]. This conformational change causes a little amount of proteolytic activity in factor XII, this is known as autoactivation.Further the surface-induced active conformationof factor XII is suggested to enhance the proteolytic conversion to*α*-factor XIIa. The gene for factor XII is located on chromosome 5q35.3.

FACTOR XIII

Factor XIII consists of two factor XIIIA subunits which is bound to two factor XIIIB subunits. It circulates in plasma as an A2B2 complex[52] . Factor XIIIA is synthesized by the monocytes/macrophages, megakaryocytes, and hepatocytes. Factor XIIIB is synthesized exclusively in the liver and kidney. The plasma

half-life of factor XIII is 10 days. Later on activation, it crosslinks and causes stabilization of fibrin clots. The crosslinking of fibrin by factor XIIIa is essential for clot formation and structure as crosslinks stabilize a clot by incorporation of the plasmin inhibitor α2-antiplasmin which makes it resistant to fibrinolytic attack by plasmin[53]. The factor XIIIA chain genehas been localized to chromosome 6 p25. The gene encoding the factor XIIIB chain has been localized to chromosome1q31. In newborns factor XIII–deficiency presents as bleeding from the umbilical cord and it is characterized by a life-long bleeding tendency . In affected women it may lead to spontaneous miscarriages[54]. When there is loss-of-function mutations in the factor XIIIA or XIIIB genes leads to a severe bleeding disorder which is rare. Acquired factor XIII deficiency arises due to the development of an inhibitory antibody that may lead to fatal bleeding if not treated.

VON WILLEBRAND FACTOR

VWF is a large multimeric glycoprotein that plays a main role in normal adhesion of platelet to components of the vessel wall . It also serves as a carrier for factor VIII[55]. It is synthesized in megakaryocytes and endothelial cells and it is stored as specialized organelles in platelets and endothelial cells. VWF multimers are released from these organelles on a stimulus or through unstimulated basal secretion from endothelial cells[56]. VWF multimers have a half-life of 8 to 12 hours . Clearance of VWF multimers takes place by means of macrophages from the liver and spleen.

Large VWF multimers are cleaved by a plasma protease ADAMTS-13 (a disintegrin and metalloproteinase with thrombospondin motifs 13).On cleavage many smaller size VWF multimers that circulate in plasma are synthesized. Reduced ADAMTS-13 activity is associated with various microangiopathies[57]. Large VWF multimers are more active than smaller multimers, because multimerscontain multiple domains that aids in the interactions between platelets, endothelial cells, and subendothelial collagen. The VWF gene is located on chromosome 12p13.3. The VWF gene is very polymorphic, as it is sometimes difficult to distinguish between the disease causing mutations and neutral gene variations. Qualitative or quantitative deficiencies in VWF cause von Willebrand disease (VWD), a mild to severe bleeding disorder. Quantitative deficiency of VWF leads to type 1 or type 3 VWD, whereas functional or qualitative defects lead to type 2 VWD[58].

THE ANTICOAGULANT PROTEINS

Control of coagulation reactions is very important to achieve normal hemostasis. Haemostasis is a host defense mechanism that responds to vascular injury. The blood coagulation factors act along with the endothelium and blood cells in particular with the platelets, to generate a protective fibrin-platelet clot which forms a hemostatic plug. For normal hemostasis, both procoagulant and anticoagulant factors must interact with the vessel wall components and cell surfaces and platelets.

The natural anticoagulants play a critical role in controlling thrombin generation[20] and are:

1. Antithrombin inhibits thrombin, FXa, IXa, XIa
2. Protein C inhibits FVIIIa and FVa
3. Protein S
4. TFPI inhibits TF/FVIIa
5. Thrombomodulin activates protein C and TAFI in acomplex with thrombin
6. Heparin cofactor 2 inhibits thrombin
7. a2-Macroglobulin inhibits thrombin and FXa
8. a1-Antitrypsin inhibits FXIa and other serine proteases
9. Protease nexin 2

Antithrombin

AT is synthesized in the liver and it circulates in plasma with a half-life Of 2.4 days[59]. AT neutralizes all procoagulant serine proteases and is present at twice the concentration of its substrates. Antithrombin is a potent inhibitorof thrombin, FIXa, FXa and FXIa[59]. AT plays an essential role as an inhibitor of coagulation .Heterozygous loss-of-function mutations leads to increased risk for thrombosis.

Protein C

Protein C was discovered in 1960. It is synthesized in the liver and circulates in plasma as a two-chain zymogen consists of 417 amino acids. It has a half-life of

6 to 8 hours[60]. Protein C is proteolytically activated by α-thrombin in complex withthe endothelial cell surface protein thrombomodulins. The mature serine protease activated protein C (APC) is formed by release of the activation peptide . Many snake venom proteases are capable of activating protein C. The protein C gene is located on chromosome 2q14.3 . Loss-of-function mutations of protein C gene cause protein C deficiency. In homozygous or compound heterozygous form this leads to life-threatening purpura fulminans at birth[61] . Heterozygous protein C deficiency leads to increased risk of venous thrombosis.

PROTEIN S

Protein S was discovered by the group of Earl Davie in 1977. It is named after the city - Seattle where it was discovered. It is a vitamin K–dependent single-chain glycoprotein of 635 amino acids and has a plasma half-life of 42 hours . It issynthesized primarily in the liver by hepatocytes, in addition ,it is also synthesized in the endothelial cells, megakaryocytes, testicular Leydig cells, and osteoblasts. Majority of protein S circulates in a bound form with the complement regulatory protein C4b–binding protein (C4BP)[60]. Remaining circulates in a free form. Free protein S serves as a cofactor for activated protein C in the proteolytic inactivation of factors Va and VIIIa. The gene encoding protein S (PROS1*)* is located on the long arm of chromosome 3 (3q11.1) Loss-of-function mutations in PROS1 gene lead to protein S deficiency.Several cases

of homozygous and compound heterozygous protein S deficiency have been described with extremely low protein S levels.

These cases are very rare and they suffer from life-threatening purpura fulminans at birth[62]. Protein S deficiency is also associated with increased risk of venous thrombosis by 10-fold.

Thrombin-Activatable Fibrinolysis Inhibitor

TAFI is the zymogen of a zinc-bound metalloprotease. It is synthesized in the liver and is proteolytically activated by plasmin or thrombin. Thrombin plays a main role in the process of clot formation and also helps in stabilization of clot by activating TAFI[63]. Activation of TAFI is increased to 1000-times when thrombin is bound to thrombomodulin. Activated TAFI protects the fibrin clot against lysis. TAFIa also has an important role in regulation of inflammation , blood pressure and wound healing[64]. The gene for TAFI is located on the chromosome 13q14. Increased TAFI levels are correlated with an increased risk of venous thrombosis.

BLEEDING IN HAEMOPHILIA:

Haemophilia A, B, C patients are deficient in F VIII; F IX and F XI respectively. These three factors are essential for conversion of F X to its activated form F X a. Deficiency of these factors block the intrinsic pathway (i.e.) contact activation pathway. Although extrinsic pathway or tissue factor pathway is active and is responsible for initial F X a generation and provides

thrombin to induce aggregation of platelets; activation of cofactors F V & F VIII in normal haemostasis; persistent haemostasis requires continuous production of F X a by the action of F IX a and F VIII a[65]; F X a production by F VIII a-TF complex is also inhibited by TFPI (Tissue Factor pathway Inhibitor). Bleeding occurs in haemophilia patients because F Xa generated by F VIIIa TF complex is inhibited by TFPI. So, intrinsic pathway must also be active for continuous production of FXa via the action of F IX a and F VIII a.

Formation of membrane bound F VIII a-F IX a complex activates F X. Absence of either activated factor leads to a similar lack of platelet surface X a activity[68]. In the presence of F VIIIa the rate of FX activation by F IX a is enhanced. Lack of platelet surface X a activity leads to decreased thrombin generation. The FXa produced by FIXa-FVIIIa is essential for generating the thrombin burst during the propagation phase of coagulation (which produces >95% of circulating thrombin), following the down-regulation of the initiating extrinsic pathway by tissue factor pathway inhibitor. FXa-FVa then converts prothrombin to thrombin, which triggers the amplification of the coagulation cascade through positive feedback to FVII, FV, FVIII, FXIII and platelet activation, eventually leading to the formation of cross-linked fibrin. So, in patients with haemophilia A & B due to decreased F VIII and F IX levels Clot formation is delayed and thrombin generation is also decreased[70].

The clot which is formed will also be friable, easily dislodged and highly susceptible for fibrinolysis[71]. This is responsible for poor wound healing and excessive bleeding in haemophilia patients .

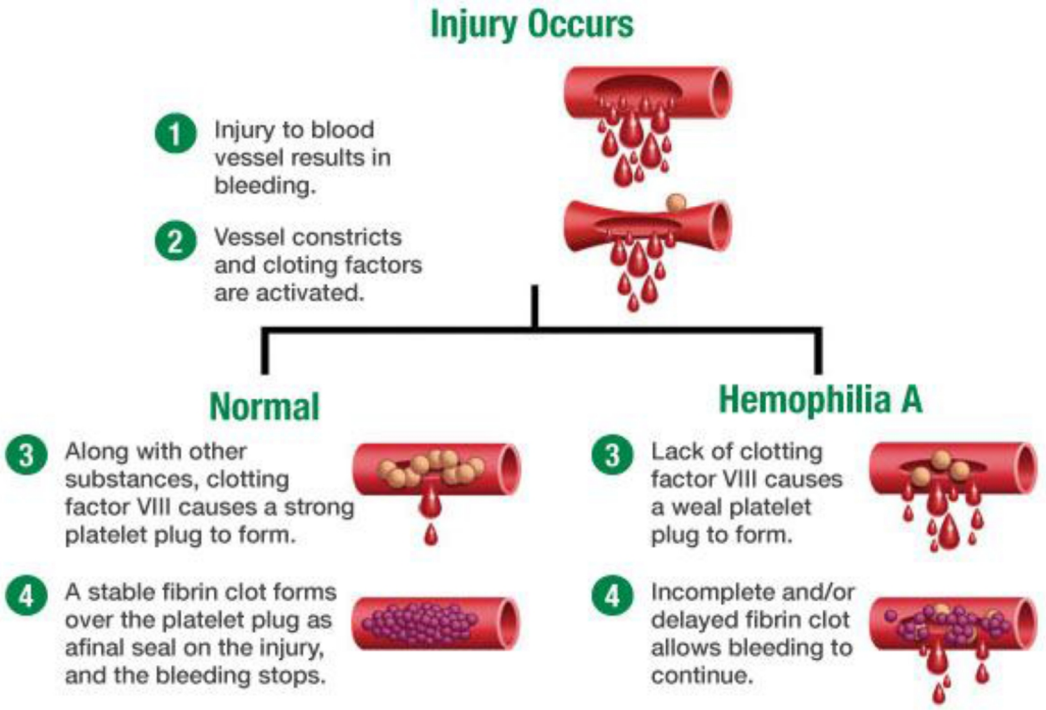


Fig: 5 Bleeding in haemophilia patients compared to normal population.

Adapted from :World Journal Of Pharmacy And Pharmaceutical Sciences Hemophilia-Inherited Bleeding Disorder: An Overview 07 March 2014

V. GENETICS

F VIII gene is located in long arm of X-chromosome. It is a large 186-kb gene with 26 exons which encodes for a polypeptide chain of 2351 amino acids[72]. They undergo post-translational modifications and release heterodimeric proteins. X-linked recessive pattern of inheritance of the disease

exclusively affects the males and females will be carriers of the disease. Male – XY: Female-XX, so affected male with non functioning F VIII gene on X-chromosome exhibits the disease and as females have another normal X-chromosome they will be carriers. Denovo females exhibit severe symptoms as male. If carrier females exhibit excessive lionisation (Inactivation) of normal X-chromosome may have low levels of F VIII and exhibit symptoms of bleeding.

Haemophilic father will not transmit the disease to his sons whereas all the daughters will be obligatory carriers.A carrier mother has 50% chance of transmitting the defective gene to a son who exhibits the disease or to a daughter who inherits the trait.

	Haemophilic Male X^h Y	
Normal X Female X	X X^h (Carrier female)	X Y (Normal Male)
	X X^h (carrier female)	XY (Normal Male)

	Normal Male X	Normal Male Y
Carrier X^h Female X	X X^h (Carrier female)	X^h Y (haemophilic Male)
	X X (Normal female)	XY (Normal Male)

Fig:6 Genetic Inheritance Pattern In Haemophilia.

Adapted From: WilliamsHematology Ninth Edition Hemophilia A And Hemophilia BMiguel A. Escobar And Nigel S. Key

In 30% of Haemophilia cases there is no family history of diseases indicating spontaneous (de nova) mutations[73]. De nova occurrence of haemophilia result from mutation in the gamete of normal male.

VI. CLINICAL MANIFESTATIONS

HEMOPHILIA A

Clinical manifestations of haemophilia depends on the level of the clotting factors. Bleeding into soft tissues, muscles and joints is the clinical hallmark of haemophilia . First bleeding episode leading to the diagnosis includes prolonged bleeding from umbilical cord, prolonged bleeding after

circumcision, after tooth extraction, after exposure to any injury or trauma ,unusual bleeding after vaccination ,unexplained and increased bleeding from any cuts or injuries, epistaxis ,bleeding gums, malena, hematuria[74]. Patients with the mild and moderate disease generally bleed after significant trauma or major surgery, those with the severe form also bleed spontaneously or after minor trauma. Patients with severe hemophilia, in the absence of preventive treatment experience on average 15 to 35 spontaneous joint and muscle bleeds per year.

Haemophilia and joint disease:

The Major clinical manifestation of haemophila is intra-articular and intramuscular bleeding. Joint disease is a disabling and common complication of severe haemophilia[75]. Recurrent bleeding into joints leads to the development of chronic arthropathy . Individuals with severe haemophilia are more likely to develop joint problems and reduced range of movement (ROM) of joints. Patients with higher frequency of bleeds, presence of inhibitors, and recent orthopaedic procedures are also associated with increased likelihood of ROM limitation[76].

Recurrent bleeding into joints and muscles can be prevented by prophylaxis (Regular replacement therapy) with clotting factor concentrates . The most commonly affected joints in patients not treated with prophylaxis are the knees (45%), followed by the elbows (30%), ankles (15%), shoulders

(3%), and wrists (2%)[77]. Therefore, these joints are often called 'index joints'.Ankle joint is the most common site of bleeding for patients on prophylaxis[78]. Reason is that current prophylactic regimens and treatment in the home makes the patients to be more active and they are able to participate in higher impact sports and activities .This renders the ankle the most vulnerable joint .

Pathophysiology

There are three stages in the development of haemophilic arthritis[79] :

1. Acute haemarthrosis
2. Chronic synovitis
3. Degenerative arthritis.

Initial bleeding into the joint causes severe pain due to increased pressure in the synovial cavity and bone marrow. Synovium lining the cavity has a limited capacity for absorbing blood .Recurrent bleeding into the joint leads to increase in a level of blood breakdown products that cannot be removed by the synovial membrane. Iron, a key component of haemoglobin seen in erythrocytes, plays a major role in inflammation of the synovium[79] . The presence of the iron-rich breakdown product haemosiderin is thought to promote the production of pro-inflamatory cytokines such as interleukin (IL)-1, IL-6, and tumour necrosis factor-alpha and the induction of genes that causes cellular proliferation such as mdm[80] . The synovium becomes increasingly vascular and hypertrophic and inflammatory cells are recruited.

This impinges between the articular surface of the joints leading to further haemarthrosis. Inflamatory mediators also leads to cartilage damage.

An acute bleed into a joint results in severe pain as the pressure in the synovial cavity and bone marrow rises, and may lead to avascular osteonecrosis (particularly in the femoral head following a bleed into the hip joint)[81]. Recurrent bleeding into joints leads to chronic synovitis , synovial damage , damage to cartilage and bone. If appropriate treatment is not given to patients with severe disease they will develop clinical symptoms such as pain, swelling, and reduced ROM by early adolescence.

Hemarthroses start early in a patient's life – usually around the time when the child begins to walk. It was found that the mean age of first joint bleeding was 1.91 years, and by the age of 2 years over 80% of the subjects had experienced at least one joint bleed[82].

Repeated hemarthrosis leads to the development of target joints, which are more prone to continuous bleeding and the development of chronic arthropathy[83].

Hematoma:

Bleeding in muscle accounts for 10-25% of hemorrhagic episodes in severe haemophilia A[74] . Muscular hematomas are considered as the major cause of disability in haemophilia patients. It can occur either spontaneously or as a result of trauma or even by post emotional stress. Around 75% of the patients with severe haemophilia A will experience hematomas during their

lifetime.The main clinical manifestations of hematoma is pain and inflammation. The severity of haemophilia correlates with the size of hematoma,involvement of fascia and muscle type[84]. After the formation of muscle hematoma, a rapid and protective muscle spasm occurs. This muscle spasm is associated with the restriction of joint movements and pain. In traumatic hematoma superficial ecchymosis and sore may be seen. The necrosis of muscle can be confirmed by the detection of keratin kinase, lactate dehydrogenase, amino-transferase andaldolase. The performance of ultra-sonography(USG), computed tomography (CT) or magnetic resonanceimaging (MRI) may help to determine the extent of hematoma[85]. Hematoma is almost always accompanied by inflammation .Intramuscular injections can cause hematoma. So its use in hemophilia patients is not usually indicated. Iliopsoas hematoma is a life-threatening condition as it is associated with the high volume of blood loss in the retroperitoneal space that may lead to death but it is a rare entity[86].

Clinical symptoms of iliopsoas hematoma includes vague pain over lower abdomen and upper thigh & features of iliopsoas is femoral nerve compression.

Measurement of Joint Health

Hemophilia is characterized by frequent intra-articular and intramuscular bleeding which causes damage to joints and soft tissues. It predisposes to joint arthropathy and musculoskeletal dysfunction. Patients with haemophilia are more susceptibile to joint hemorrhage. This indicates that the routine

measurement of joint health is an important step in the clinical management and assessing the treatment outcome i.e the efficacy of treatment. The current standard for diagnosis and assessment of arthropathy is the World Federation of Hemophilia (WFH) Physical Evaluation Scale. This has served as an important measure of joint outcome in patients with haemophilia. The Functional Independence Score in Hemophilia (FISH) is a performance-based assessment tool used to measure the patients functional ability ; mostly used in patients with severe hemophilia[86]. A significant decrease in functional ability was observed in patients with severe hemophilia, especially for those activities involving weight-bearing demands like locomotion and step climbing.

In FISH scoring system each patient is evaluated in seven activities under three categories: self-care (grooming and eating, bathing, and dressing), transfers (chair and squat), and locomotion (walking and step climbing). Each activity was graded from 1 to 4 according to the amount of assistance required to perform the activity with total scores ranging from 7 to 28[88]. The aim of treatment of hemophilia with factor replacement therapy is to minimize structural damage to joints and maximize patients' functional independence and quality of life.

The FISH scoring system may be extremely useful in the clinical practice in the absence of imaging methods such as magnetic resonance imaging (MRI), which are considered very sensitive to detect early joint damage[87].

Life-Threatening Hemorrhages

Life-threatening bleeds such as intracranial hemorrhages (ICH) are relatively rare. But if present can cause a significant morbidity. It can present with painful and prolonged headache, vomiting, diplopia, convulsions etc[89]. In the 1960s, Before the introduction of newer therapeutic options, a 75% of intracranial hemorrhage caused death; however, nowadays this is reduced to about 30%[90]. It occurs more frequently in children and young adults as they are involved in more physical activities and has higher possibility of direct trauma to the head. . During the neonatal period, ICH affects 3.5-4.0% of all children with haemophilia, with the majority being trauma-induced. After the neonatal period ICH affects 3-10% of patients not receiving preventative treatment and accounts for approximately 20% of all deaths in patients with haemophilia. Early diagnosis and early commencement of treatment can decrease the mortality and prevent more complex neurological sequelae[91] . Prior to the wide availability of treatment, one third of all deaths in patients with haemophilia resulted from ICH.

Gastrointestinal Bleeding:

The occurrence of gastrointestinal bleeding is rare in hemophilia. It can occur as spontaneous bleeding or secondary to other common causes of GI bleeding. Use of NSAIDS can increase the risk of upper GI bleed in these patients.

Helicobacter pylori infection can increase the frequency ofGI bleed in patients with haemophilia[92].

Hematuria:

Hematuria is one of the troublesome clinical feature in haemophilia patients. Patient presents with flank pain and sudden abdomen pain due to hydronephrosis or ureteral obstruction secondary to hemorrhage[74].

Bleeding On Minor Procedures:

Circumcision and dental extraction are the two invasive procedures that poses challenge to the hemostasis system. Post circumcision bleeding is common among babies with hemophilia who do not receive a sufficient amount of coagulation factor prior and post-procedure. Sometimes it may be the first bleeding manifestation of a baby with a mild haemophilia[93].So in neonates or babies with a negative past history of haemophilia and also in babies with positive family history of haemophilia, circumcision practice must be delayed until the primary screening test results are known.

COMPLICATIONS OF HAEMOPHILIA A

Development of Chronic Arthropathy

Recurrent joint bleeding leads to the development of chronic arthropathy, a major complication of hemophilia that is associated with pain, loss of function, long-term impairments, and reduced quality of life[94]. In developing nations such as India where patients with hemophilia have limited access to treatment, there is widespread disability from recurrent joint bleeds. Morbidity from joint impairment increases significantly with advancing age[95].

The number of joint bleeds is a significant predictor for the development of haemophilic arthropathy .

The vicious cycle – of repeated bleeding and the development of target joints – eventually leads to the loss of joint space, complete erosion of articular cartilage, and permanent disabling haemophilic arthropathy[96].

Treatment of Hemophilic Arthropathy

Therapeutic options for established haemophilic arthropathy include physical therapy, rehabilitation techniques and surgical procedures such as medical synovectomy using chemical or radioactive substances, surgical synovectomy by open or arthroscopic technique, or total joint replacement[97].Using a rating scale for evaluating changes following total knee replacement in patients with hemophilia, it has been suggested that patients with multiple affected joints do not demonstrate major improvements in post-operative scores compared to the response seen in patients with isolated joint problems. Improvement in pain is seen in these patient but overall range of movement may not change following joint replacement[98].

VII. DIAGNOSIS

CLINICAL LABORATORY TESTS FOR HAEMOPHILIA

The diagnosis of haemophilia is suspected when the patient presents with symptoms like spontaneous bleeding to joints, muscles and soft tissues, hemarthrosis, deep muscle hematomas. Some of them can present with positive

family history. A confirmed diagnosis can be made only based on coagulation factor assay . The following tests can be done.

Tests available for the diagnosis of haemophilia:

Most of the bleeding disorders have clinical similarities and it is of atmost importance to identify exact etiology to give more accurate therapy for recovery.

1) Basic screening tests for hemophilia

a) Bleeding time

Bleeding time is the time interval between making a small standard cut and the moment the bleeding stops. Bleeding time measures platelet adhesion and activation so, it is said to be normal in haemophilia. In a study by M.Elaine eyster et.al.,[99] it was found out that mean bleeding time of haemophilia patients were prolonged than the normal controls. This prolongation was not associated with the severity of the disease, use of NSAIDs or transfusions.

b) Platelet count

In the study of bleeding tendency of patients with haemophilia platelets play a less important role. Platelet function assessed in terms of primary hemostasis is generally considered to be normal.Haemophilia patients generally have normal range of platelet count. In a study by Esther R. van Bladel et. al.,[100] it is found out that Haemophilia A is associated with higher basal level of platelet activation. It is indicated by higher P-selectin expression on the platelet surface and higher plasma concentrations of soluble platelet activation markers like

PF4, CXCL7 and RANTES[100]. Severe haemophilia patients in whom platelets are in a pre-activated state have lower factor VIII consumption .

Activated Partial Thromboplastin Time

It is called as "activated" PTT because the reagent used for APTT assay contains a negatively charged surface that accelerates the rate of the reaction. The test sample is collected with 3.2 g% sodium citrate as anticoagulant. The ratio of anticoagulant to whole blood is 1 part anticoagulant to 9 parts whole blood[101]. First step in APTT assay is anticoagulated patient plasma is incubated with a mixture of a negatively charged surface, phospholipid for several minutes. After incubation period the sample is recalcified by addition of excess of calcium chloride. Then the time required for clot formation is measured.

Sodium citrate is used as an anticoagulant for APTT assay because it is a reversible chelator of calcium which prevents the activation of coagulation proteins. The APTT assay is used to assesses the coagulation proteins involved in the intrinsic system and common pathways. All coagulation factors which are involved in the intrinsic system like factor XII, XI, IX,VIII , prekallikrein and high molecular weight kininogen are measured in the assays using APTT as its platform[29].

Prothrombin Time:

The PT assesses the coagulation proteins involved in the extrinsic pathway and common pathway .Test sample is anticoagulated with 3.2% sodium citrate[101] .

Anticoagulated patient plasma is incubated with tissue thromboplastin (recombinant human or isolated animal tissue factor) for several minutes. In the next step citrated plasma mixture is recalcified by the addition of excess $CaCl_2$. Then the time required for clot formation is measured. PT is used to assesses the coagulation proteins of the extrinsic system and common pathway[29].

International normalized ratio (INR)

INR is calculated by the ratio of patient PT divided by geometric

mean normal PT for the local laboratory.

2) Correction studies with factor deficient plasma

Correction studies are performed to determine the causes of prolonged coagulation time. The causes include factor deficiency, presence of circulating anticoagulants or inhibitors or presence of lupus anticoagulant. Mixing or correction studies are done using pooled normal plasma (PNP). It is performed by mixing 1:1ratio of patient's plasma with the normal pooled plasma. After mixing, the PTT is repeated and the PTT result should decrease and come close to the reference value, the reduction should be at least less than 50% of the clotting time[102]. If the PTT does not return to the reference limits, further evaluation must be done for presence of heparin, inhibitor or lupus anticoagulant[103]. Correction studies can be done with FVIII/FIX-deficient plasma to identify the particular factor deficiency if factor assay is not available.

3) Factor assays

Factor VIII activity can be assessed using 3 methods[104]. They are

- 1-stage clot-based assay
- chromogenic assay
- immunoassay

1-stage clot-based assay

It is an one-stage assay based on APTT. One stage assay measures the ability of a patient plasma to decrease the APTT of a FVIII deficient plasma [105]. The FVIII deficient plasma and patient sample are pre incubated with the APTT reagent and calcium chloride is added to it , as calcium promotes fibrin clot formation, which is the endpoint of the APTT. FVIII concentration in the patient sample is the rate-limiting determinant of the clotting time in this APTT assay. This result is compared with the standard curve generated from samples with known FVIII activities . A line of best match is created for the standard curve, and a second line of best fit is created for the patient data points (each patient is tested at a minimum of three different dilutions). The two best fit lines should be parallel unless a nonspecific inhibitor is present in the patient sample FVIII- and FIX-deficient plasma containing < 1 IU/dl of FVIII- and FIX clotting factors and normal levels of other clotting factors are taken. Either commercially available or locally prepared reference/calibration plasma is made according to WHO international standard. The reference plasma and the test sample are made into at least three different dilutions to obtain a valid assay. The precision of the test is reduced when a single dilution of test sample is used[27]. Important issues in doing mixing

studies are the ratio of patient plasma to normal plasma which ranges from 1 : 1 to 4 : 1 and the time of incubation from mixing of plasma to assay either done immediately or after 2 hours. , Chang et al, said that when the patient plasma and normal plasma were incubated at 37° C for 1 hour before assay at a ratio of 1 : 1 or 4 : 1 (patient :normal) the sensitivity and specificity increases[106].

Chromogenic assay

The chromogenic FVIII activity assay is done as a two staged assay. In the first stage patient plasma which contains an unknown amount of functional FVIII is added to a reaction mixture . Reaction mixture consists of thrombin or prothrombin, FIXa, FX, calcium, and phospholipid. This reaction immediately produces FVIIIa, which on action with FIXa activate FX. When the reaction is stopped, FXa production is assumed to be proportional to the amount of functional FVIII present in the sample. In the second stage FXa is measured through cleavage of a FXa specific peptide nitroanilide substrate. P-nitroaniline is produced, which gives a color that can be measured photometrically by absorbance at 405 nm. The color produced is directly proportional to the amount of functional FVIII present in the sample based on a standard curve[107][108].

Immunoassays

Monoclonal antibodies against human factor VIII are used to measure FVIII:CAg. The antibodies directed against FVIII are produced commercially on large scales. This is used to measure FVIII:CAg by two stage ELISA[109].

MANAGEMENT OF HAEMOPHILIA:

FACTOR REPLACEMENT THERAPY

The prevention or treatment of joint bleeding in patients with hemophilia involves factor replacement therapy through the intravenous infusion of the deficient clotting protein[110]. Replacement factor products can be blood-derived or recombinant. Currently, there are two main approaches for treating haemophilia patients[111]. They are

- Intermittent replacement therapy ("on-demand")
- Prophylactic therapy.
  - Primary prophylaxis.
  - Secondary prophylaxis.

Types of Clotting Factor Product

There are two types of replacement factor products used to treat hemophilia A. They are plasma-derived FVIII and recombinant FVIII (rFVIII). Plasma-derived FVIII is associated with hepatitis C and human immunodeficiency virus (HIV) infections[112].

Intermittent Factor Replacement Therapy

Intermittent or on-demand therapy refers to the infusion of clotting factor at the time of a bleeding episode for the purpose of stopping the bleed and the subsequent development of a target joint and chronic arthropathy. The standard treatment for acute joint bleeds is the infusion of 30 U/kg of FVIII at the time of bleeding. More recently enhanced episodic replacement has been practised for

treatment of joint bleeding in patients receiving on-demand treatment or of breakthrough bleeding in those on prophylaxis – 40 U/kg at the time of bleeding, and repeated at a dose of 20 U/kg on the first and third days following the bleed[113].

Prophylactic Therapy

Prophylactic therapy can be classified as either primary or secondary.

Primary prophylaxis:

Primary prophylaxis refers to the regular infusion of clotting factor before the onset of joint damage in order to prevent recurrent bleeding.

It is started after the first but before the third joint bleed. It is a preventive therapy[115]. Starting primary prophylaxis at an early age tends to decrease the incidence of severe haemophilic arthropathy. There are studies indicating that children who were started on prophylaxis before age of 3 had a better clinical outcome in terms of arthropathy[114] . The dose recommendations for primary prophylaxis in haemophilia A is 25-40 IU/kg three times weekly starting at the age of 1-2 years and for haemophilia B same dose 25- 40 IU/ kg two times weekly. The basis of this regimen is to maintain the trough level of the deficient clotting factor >1% so that severe haemophilia is converted into mild haemophilia.

Secondary prophylaxis :

Secondary prophylaxis is the regular factor infusion in patients with existing joint disease. The goal is to decrease recurrent bleeding and to delay

the progression of irreversible joint damage. Secondary prophylaxis cannot reverse the changes which have already occurred due to chronic arthropathy. The benefits of secondary prophylaxis lies in the fact that it reduces the bleeding episodes, number of hospital admissions and absence from school or work and also by decreasing the joint damage progression. Patients treated with secondary prophylaxis have low number of joint bleeds at the expense of increased consumption of clotting factors[115]. In a study published by Rodriguez and Hoots it was found out that the patients treated with on demand therapy had 3.2fold increase in the frequency of joint bleeds.

Due to the inability of secondary prophylaxis to control the progression of some joint bleeding and arthropathy, primary prophylaxis is the recommended therapy in many parts of the world.

Full-dose prophylaxis is the infusion of 25-40 U/kg on alternate days starting between the ages of 1-2 years. The aim of full-dose (Malmo) prophylaxis is to keep the trough level of FVIII above 1% of normal so that the severe form of the disease is converted to a milder form. The rationale for this approach is based on the observation that patients with moderate hemophilia rarely develop chronic arthropathy .There are studies suggesting that prophylaxis leads to reduced pain, improved physical functioning and better health-related quality of life.

Complications of Treatment

While prophylaxis appears to be the most effective therapy to prevent or limit joint disease in hemophilia, it presents the challenge of reliable venous access when started at a very early age (1-2 years of life) and it is very expensive.

Venous infusion of factor VIII

Primary prophylaxis is most beneficial when started at an early age, before recurrent joint bleeding has begun. However, venous access may be difficult in young children, thus requiring the insertion of an indwelling central venous catheter (CVC) for the frequent administration of FVIII. These devices are associated with a high risk of infection, thrombosis and mechanical failure[116].

Viral infections

It occurs due to transfusion-induced viral infections with blood-derived factor concentrates.

Hepatitis C infections affected almost all hemophilia patients receiving pooled concentrates resulting in significant morbidity and mortality.[23] HIV also caused devastation in the global hemophilia population. These infections tends to diminish the quality of life in haemophiliac adult patients in terms of "physical capacity", "pain", "general healh", "mental health"[112]. Recent advancements in viral inactivation methods and the availability of safe recombinant products have allayed earlier concerns and primary prophylaxis is

now recognized as standard of care for young boys and adults with severe hemophilia A.

Phenotypic Heterogneity In Severe Hemophilia A

The classification of hemophilia based on FVIII activity correlates well overall with clinical severity. However, FVIII activity is not always predictive of bleeding severity and musculoskeletal outcome[117]. Within cohorts of patients with severe hemophilia A, considerable heterogeneity has been observed. Despite having similar circulating FVIII levels, approximately 3-10% of these patients have a clinically mild disease with infrequent spontaneous joint hemorrhage and minimal joint damage[118].

FVIII Pharmacokinetics

FVIII pharmacokinetics, e.g. the distribution, metabolism and elimination of FVIII, likely play an important role in the clinical management of hemophilia A. FVIII pharmacokinetics assessments are sometimes used in optimizing dosing schedules and inhibitor management. The heterogeneity of FVIII pharmacokinetics in patients with hemophilia is to be considered for treatment. FVIII half-life has been shown to vary from 6 to 28.8 hours in patients with severe haemophilia[117]. Dose tailoring based on individual pharmacokinetics aims to maintain a particular trough level of FVIII.

Other treatment options

Desmopressin

Desmopressin causes a transient increase in factor VIII in normal persons and in patients with mild to moderate hemophilia. It can be administered by either intravenous route,subcutaneous or as nasal spray[119]. The maximum response of DDAVP occurs only after 30 to 60 mins of administration. The mechanism by which DDAVP increases Factor VIII is unknown. It can be used in patients with mild or moderate haemophilia A and in haemophilia carriers with baseline factor VIII levels are less than 0.5 U/mL, When DDAVP is administered repeatedly it results in tachyphylaxsis and decreased response[120] .

Antifibrinolytic Agents

Antifibrinolytic agents, like ε-aminocaproic acid (EACA) and tranexamic acid are used to enhance hemostasis in patients with haemophilia A. They are administered either orally or as continuous i.v administration[121]. It is used as adjunctive therapy for mucous membrane bleeds, dental procedures etc. It is contraindicated in hematuria as the lysis resistant clots may cause obstruction of the ureters.

Fibrin glue

Fibrin tissue adhesive is used as an adjunctive therapy to factor VIII. It contains fibrinogen, thrombin, and factor XIII[122]. Mechanism of action is that when fibrinogen– factor XIII mixture is placed on the injury site it reacts with human thrombin solution containing calcium and a crosslinked

fibrin clot is formed. It is useful in dental procedures, to control bleeding from surgical wounds etc.

Fresh frozen plasma (FFP)

FFP contains all the coagulation factors and is sometimes used to treat coagulation factor deficiencies. Cryoprecipitate is preferable to FFP for the treatment of hemophilia A[123]. One ml of fresh frozen plasma contains 1 unit of factor activity.

Cryoprecipitate

Cryoprecipitate is prepared by slow thawing of fresh frozen plasma (FFP) at 4°C for 10-24 hours.It appears as an insoluble precipitate and is separated by centrifugation. It contains FVIII at a concentration of about 3-5 IU/ml , VWF, fibrinogen, and FXIII but not FIX or FXI. The supernatant is called as cryo-poor plasma and contains other coagulation factors such as factors VII, IX,X, and XI.

Cryoprecipitate and FFP are not subjected to viral inactivation procedures so, they are not preferred by world federation of haemophilia[124].

Gene therapy

It is the replacement of a defective gene sequence with a corrected sequence to eliminate the disease for the lifetime of the patient. Gene addition methods in which a corrected copy of the defective gene is added without removal of the error-containing defective genomic sequence. Adenovirus and retrovirus are

used as vectors for gene transfer. A significant progress is seen in the field of gene therapy for haemophilia [125].

INHIBITORS IN HAEMOPHILIA

Haemophilia patients are deficient in a coagulant protein that is essential for normal blood clotting. Respective coagulation factors are given as replacement therapy by intravenous route. Alloantibodies called as inhibitors develop to the intravenous anti-hemophilic factor products given to stop or prevent a bleeding episode[126]. These inhibitors neutralise the clotting activity of exogenous coagulation factor given as replacement therapy. It is currently the most serious complication of the treatment of haemophilia. . People with haemophilia and an inhibitor are at increased risk of hospitalization for a bleeding complication.The costs of treatment and hospital care in patients with inhibitors have been reported to be 2 to 10 times greater compared with those without an inhibitor[127].

Factor VIII inhibitors are antibodies mostly Immunoglobulin-IgG belonging to subclass IgG4[128]. They are almost always alloantibodies, some of the mild hemophilia patients may develop auto antibodies. These antibodies may interfere with the interaction of Factor VIII with other hemostatic components.

Incidence of inhibitor development :

Inhibitors develop in around one-third of previously untreated

patients with severe haemophilia A usually within the first 10–15 days of treatment with replacement factor concentrates[129]. The risk of inhibitor development then decreases, becoming almost negligible in previously treated patients who have been exposed to FVIII concentrates for more than 50–150 days[130]. But , the risk never disappears. It persists throughout one's life, and shows a slight increase in incidence among the elderly patients. The incidence of Inhibitor development is around 20-30% of patients with severe hemophilia A[131]. Frequency of Inhibitor development is around 40% in patients with nonsense mutations. Genetic influence was implicated by observations of an increased incidence of inhibitor development within families and an even higher incidence among twin brothers[132].

Genetics of inhibitor development :

Inhibitor development in individuals may be due to complex interaction between Genetic and Environmental factors. Genetic factors include F8 gene mutation, patients with large deletions, non-sense mutation and Intron 22 inversion had 7-10 fold higher risk of developing inhibitors than patients with small deletions, insertions, missense mutation and splice site mutations[133]. There are polymorphisms of immune regulatory genes like TNF-α which may increase inhibitor risk or CTLA-4 which reduces risk of Inhibitor development. Non genetic factors like products released from injured tissues acts as alarm signals to the immune system, recognizing them as non-self. So, this indicates

that only presentation of exogenous F VIII/FIX may not be sufficient for initiating an immune response, in addition to exogenous F VIII/FIX it also requires danger signals like severe bleeds; trauma; surgery with major tissue injury. The foreign antigen is intensively presented to the T and B lymphocytes and an upregulated immune response occurs[134].

Regular administration of low doses of F V III/ IX as prophylaxis allows the immune system to tolerate the foreign protein whereas risk of inhibitor development increases in patients receiving intensive treatment in the initial phase due to severe bleeds or surgery.

An Italian study shows 70% reduction of inhibitor risk in children who were started on prophylaxis at a median age of 35 months compared to children who are receiving on-demand treatment.

Risk Factors for Inhibitor Development:

1) Disease Severity[135]:

- 80% of Hemophilia A Patients with inhibitors have < 1% of factor VIII activity.

2) Exposure to F VIII concentrates[135]:

- Majority of high titre inhibitors develop after < 90 days of exposure to F VIII.

3) Genetic Factors[135]:

- Family history of inhibitors development

- Negative correlation with HLA CW5 antigen

- Molecular Defects-Inversion and crossing-over defect in Intron 28, gene deletions and nonsense point mutation resulting in patients without F VIII antigen.

4) Method of purification of F VIII concentrate[135].

Inhibitor Testing:

Bethesda assay is used for Inhibitor testing[136], now Nijmegen Modification of Bethesda assay is used where pH of the sample over 2 hours incubation period is controlled[137].

F VIII inhibitors are temperature & time dependent . Bethesda assay is based on the fact that prolonged APTT in a hemophilia patient without inhibitor is corrected when mixed as 1:1 with normal plasma even after incubation at 37^0C for 1 to 2 hours. In contrast APTT of the hemophilia patient with inhibitor in 1:1 mixture with normal plasma is prolonged after incubation at 37^0C for 1-2 hours[138].

The Bethesda assay was improved by the Nijmegen modification . In this modification the normal pooled plasma is buffered with 0.1 M imidazole to

pH 7.4. This is done to prevent pH shift during incubation and replacing the buffer as a control with hemophilic or immune-depleted, FVIII-deficient plasma, which prevents dilution of the protein content in the mixture with normal plasma[139].

The Nijmegen modification has increased specificity without affecting the sensitivity. It was confirmed by a Canadian study which showed a reduced number of false positive assay results with the Nijmegen assay .

Hence, the Nijmegen assay is recommended as the standard

assay for FVIII inhibitor testing by the International Society of

Thrombosis and Hemostasis Factor VIII/IX Scientific Subcommittee[140].

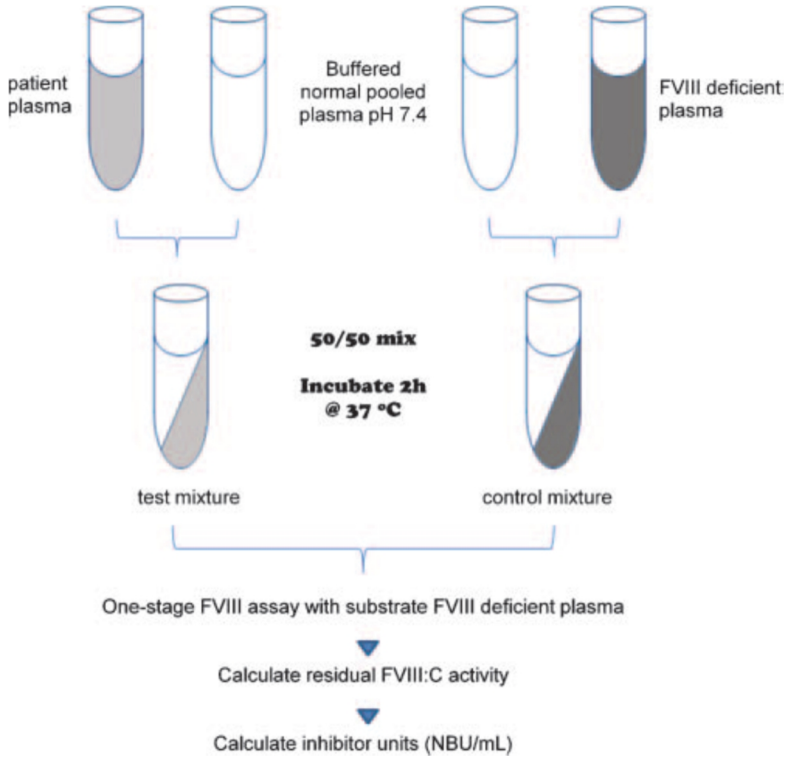


Fig: 7 A schematic representation of the Nijmegen assay

Adapted from: Factor VIII Inhibitor Assays: Methodology, Shortcomings,and Challenges M van Geffen, M Dardikh1 and B Verbruggen

TREATMENT OF HAEMOPHILIA WITH INHIBITORS:

Haemophilia patients with inhibitors are treated with bypassing agents like activated prothrombin complex concentrates (aPCC) (FEIBA®) and recombinant activated factor VII (rFVIIa) (NovoSeven®, Novo Nordisk)[141]. Mechanism of action is that these products overcome the haemostatic interference of the inhibitor. But these substitutes are not comparable with that of factor concentrates. Immune Tolerance Induction or ITI is a modality of management of severe haemophilia with inhibitors in which elimination of inhibitors is tried by repeated high doses of factor VIII concentrates infusion[142].

rFVIIa :

rFVIIa is produced by baby hamster kidney (BHK) cells .BHK cells express the cloned human F7 gene. A new formulation of rFVIIa has been produced with sucrose and L-methionine. The advantage is that the product can be stored at room temperature before reconstitution. Mechanism of action of rFVIIa is by promoting haemostasis by activating FX directly on the platelet surface, this bypasses the tenase complex[143]. The consequent increase in thrombin generation enhances platelet aggregation, leads to the full activation of thrombin-activatable fibrinolysis inhibitor and FXIII, and leads to the formation of a tight fibrin plug. The half-life of rFVIIa is 2.3 h in adults, and it is shorter in children .

FEIBA :

FEIBA is Factor Eight Inhibitor Bypassing Agent . It is produced from the vapour-heated concentrate of plasma-derived, vitamin K-dependent clotting factors (FII, FVII, FIX, and FX) in both zymogen and active forms. Its mechanism of action is multifactorial. FII and activated FX are found out to be the most important components.

In patients with low-titre inhibitors (<5 Bethesda units [BU]), haemostasis is achievable with higher than- normal doses of factor which overcome the inhibitor action. In patients with high-titre inhibitors (>_5 BU), bypassing agents like FEIBA or rFVIII are needed to achieve haemostasis[144].

MATERIALS AND METHODS

PLACE OF STUDY

Haemophilia day care centre Tirunelveli Medical College Hospital.

DURATION OF STUDY

March 2017 to September 2018(18 months).

STUDY DESIGN

Prospective study

SAMPLE SIZE

35 cases

ETHICAL COMMITTEE APROVAL

This study was conducted after getting approval of the institutional ethical committee. A copy of approval is enclosed.

SELECTION OF STUDY POPULATION

Inclusion criteria for cases

Known cases of haemophilia A, haemophilia B.

Newly diagnosed cases of haemophilia during the study period.

Exclusion criteria for cases

Patients with bleeding disorders other than haemophilia.

METHODOLOGY

Patients are explained about the research project & consent is obtained.

a)History:

Detailed history regarding onset and progression of the disease, family history-relatives suffering from the disease are interviewed through face to face interview using a structured questionnaire. Age of diagnosis of the disease was asked. Treatments undertaken by the patient were noted. Time of first administration of Factor VIII and Factor IX concentrates was asked. Frequency & time interval between Factor VIII and IX administration was noted. Other treatments undertaken like desmopressin , blood transfusion, cryoprecipitates, fresh frozen plasma. Questions regarding complications of treatment with blood and blood products like transfusion reactions, transfusion transmissible infections were asked. Number of admissions of the patient in the day care centre during the study period, time interval between each admission were recorded. All the patients were registered in the haemophilia society and graded according to the factor levels into mild , moderate and severe haemophilia. Details regarding symptom complex with which patients are admitted was noted

. Physical examination, Disability assessment are done using FISH (Functional Independence Score in Haemophilia) .

b)Physical examination

It is done to look for any swelling ,muscle atrophy, crepitus on motion ,joint deformities, Ankylosis, gait change.

c)Disability assessment

Every patient is examined for 7 activities under 3 categories . It includes

- self-care (eating and grooming, bathing and dressing),
- transfers (chair transfer and squatting) and
- locomotion (walking, stair climbing).

Each activity was graded from 1 to 4 according to the level of assistance required for each activity. Total score was calculated and statistical analysis is done .

d)Laboratory parameters

Under aseptic precautions blood sample was collected with minimal stasis from the antecubital vein using dry sterile disposable syringe and needle. Venous samples for complete blood count and coagulation analysis was collected.CBC-sample: 2ml of blood with EDTA as anticoagulant run in semi automated 5 part counter.Coagulation profile- 2ml of blood with 3.2% Tri sodium citrate as anticoagulant run in semi automated analyser. Inhibitor levels were done in

selected set of patients on long term treatment at various laboratories. The results were obtained from the patients medical records.

OBSERVATION AND RESULTS

This study was done on haemophilia patients admitted at the day care centre of Tirunelveli medical college hospital from a period 1.3.2017 to 30.8.2018.

a)Age wise distribution of the cases

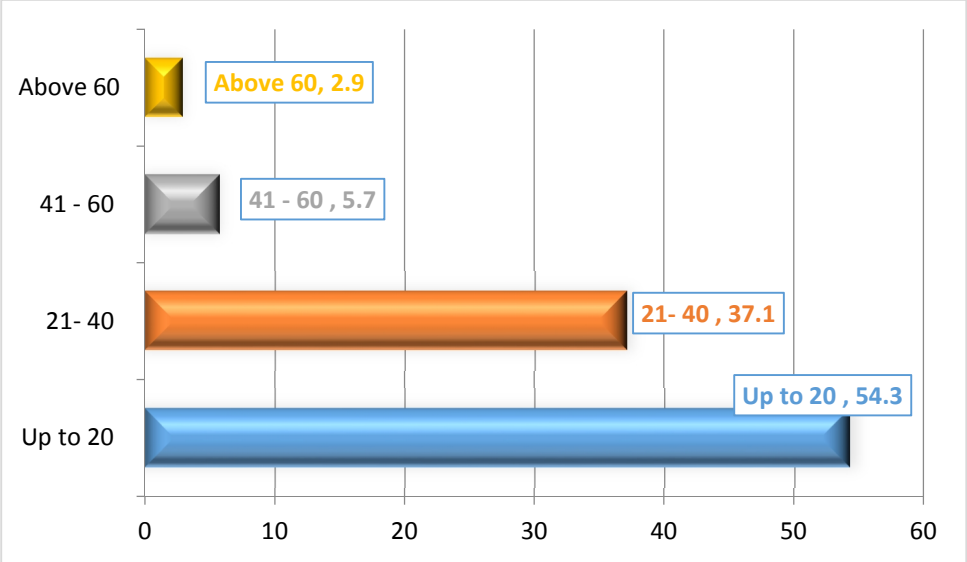
Out of 35 cases with Haemophilia it was noted that 19 patients were below the age group of 20 years; 13 patients were in the age group of 21-40 years; 2 patients were in the age group of 41-60 years; 1 patient was in the age group of above 60 years. Mean age of the study population is 25.42 years (n=35).

(Table – 1,Chart- 1).

Table – 1: Age wise distribution of the cases

Age In Years	No. Of Cases	Percentage
Up to 20	19	54.3
21- 40	13	37.1
41 - 60	2	5.7
Above 60	1	2.9

Chart- 1: Age wise distribution of the cases



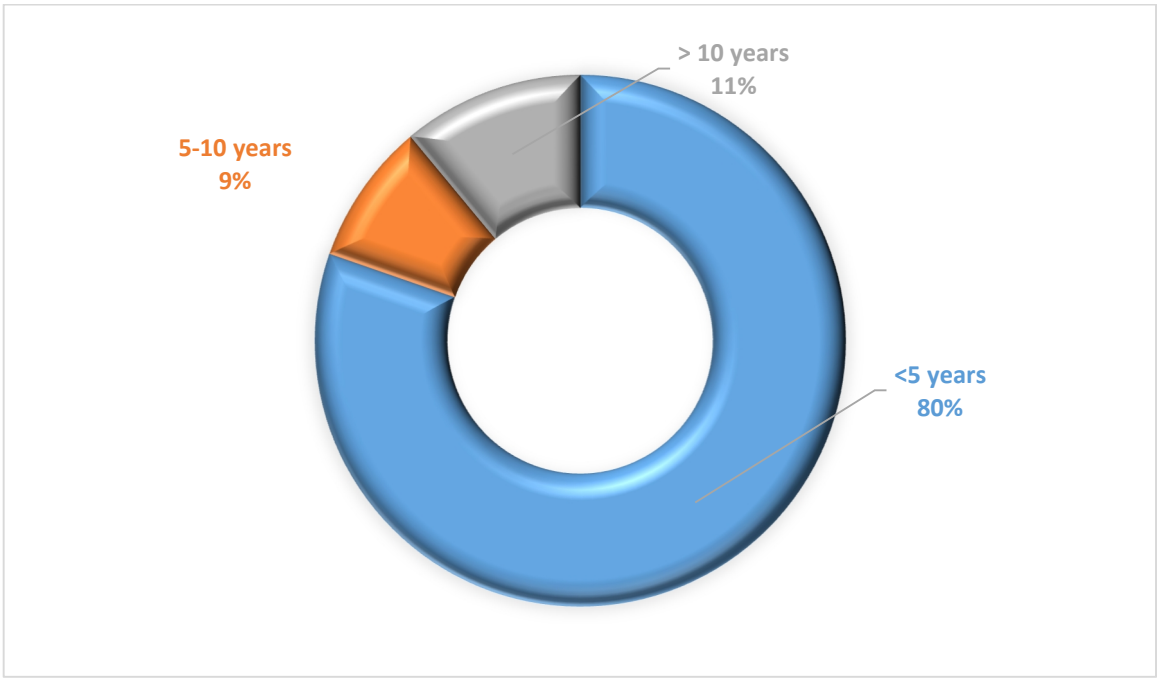
b)Distribution of the cases based on age at first diagnosis of the disease

Out of 35 cases, 28 cases were dignosed before the age of 5 years; 3 cases were diagnosed between the age group of 5- 10 years; 4 cases were diagnosed >10 years of age. (Table – 2,Chart- 2)

Table – 2: Distribution of the cases based on age at first diagnosis of the disease

Age at first diagnosis of the disease	No. of cases	Percentage
<5 years	28	80
5-10 years	3	8.6
> 10 years	4	11.4

Chart – 2: Distribution of the cases based on age at first diagnosis of the disease



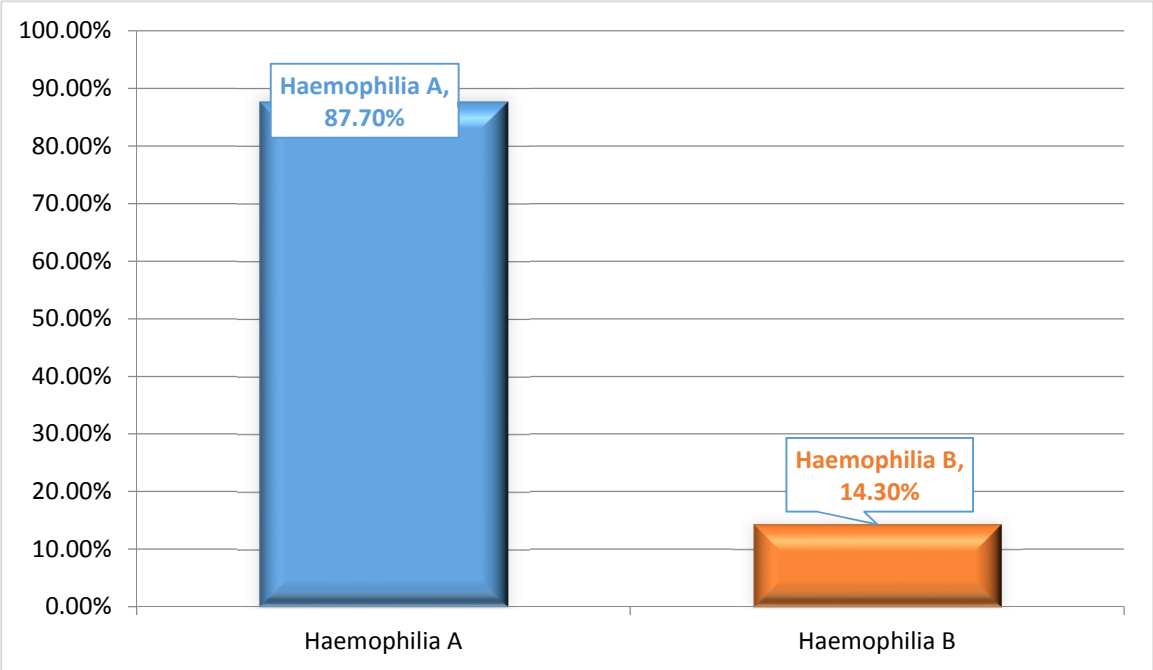
c) Case distribution based on type of haemophilia

Out of 35 cases of Haemophilia, 30 cases were HaemophiliaA and 5 cases were haemophilia B. (Table – 3 & Chart – 3)

Table – 3: Case distribution based on type of haemophilia

Types of Haemophilia	No. of cases	Percentage
Haemophilia A	30	87.7 %
Haemophilia B	5	14.3%

Chart – Type of haemophilia based case distribution



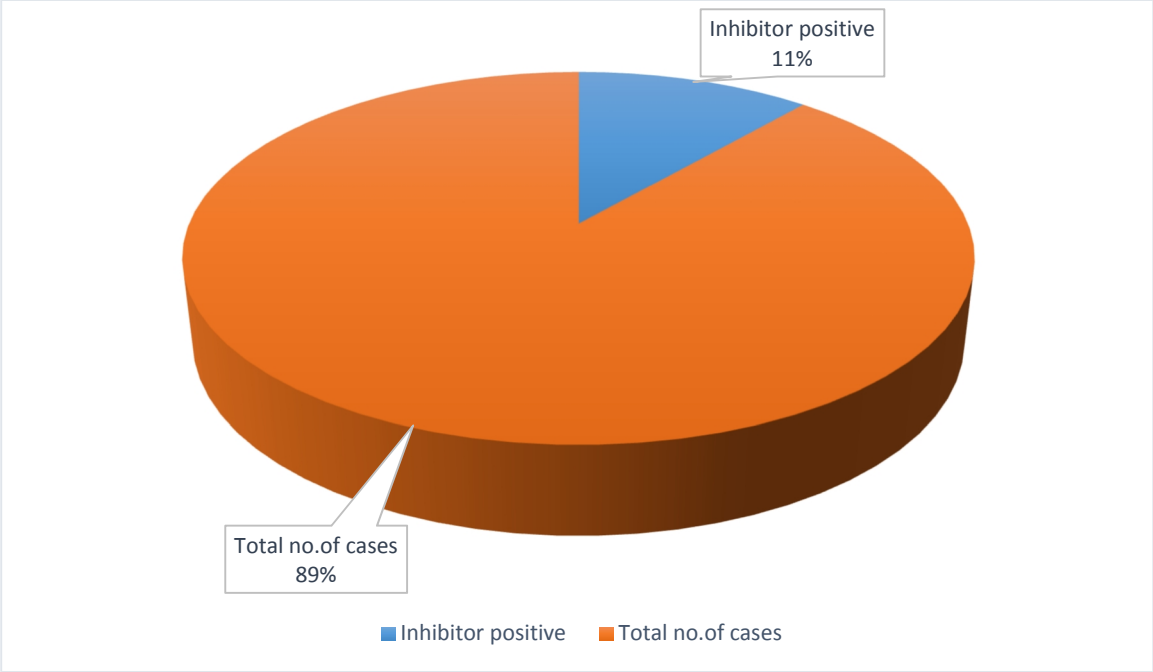
d)Case distribution based on inhibitor status

Out of 35 cases of Haemophilia 4 patients were positive for inhibitors (Table – 4,Chart- 4).

Table -4 Case distribution based on inhibitor status

Total no. of cases	Inhibitor positive	Percentage
35	4	11.4%

Chart – 4 Case distribution based on inhibitor status



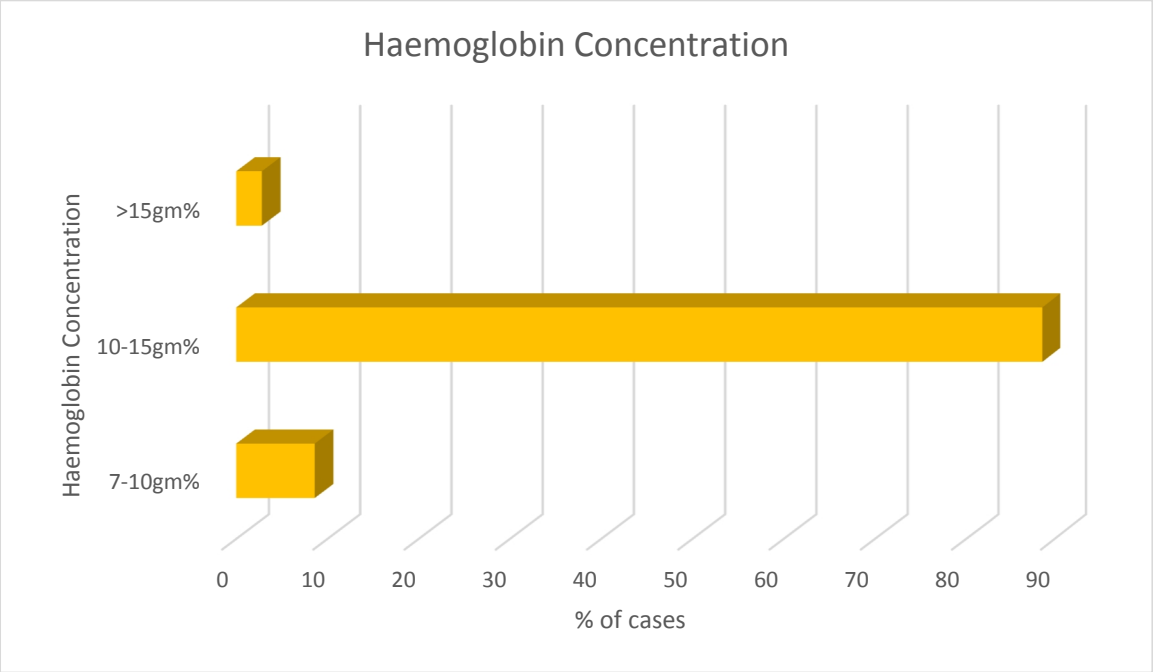
e) Distribution of the cases based on the haemoglobin concentration

Out of 35 cases 3 cases had haemoglobin concentration between 7-10gm% , 31 cases had haemoglobin concentration between 10gm-15% and 1 patient had haemoglobin concentration of >15 gm%. (Table – 5,Chart- 5)

Table – 5: Distribution of the cases based on haemoglobin concentration

Haemoglobin Concentration	No. of cases	Percentage
7-10gm%	3	8.6%
10-15gm%	31	88.6%
>15gm%	1	2.8%

Chart- 5: Distribution of the cases based on the haemoglobin concentration



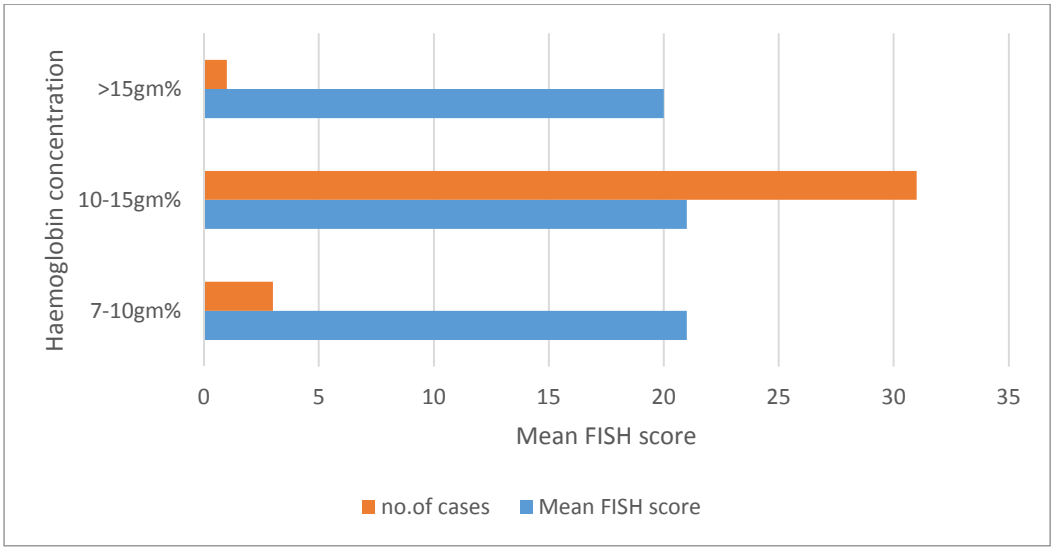
f)Distribution of the cases based on the haemoglobin concentration and the severity of the disease

Out of 35 cases, 3 cases in the haemoglobin range of 7-10 gms% had a mean FISH score of 21; 31 cases in the haemoglobin range of 10-15 gms% also had a mean FISH score of 21; 1 case with haemoglobin >15 gms% had a mean FISH score of 20.(Table – 6,Chart- 6)

Table – 6:Distribution of the cases based on the haemoglobin concentration and the severity of the disease

Haemoglobin Concentration	No. of cases	Mean FISH score
7-10gm%	3	21
10-15gm%	31	21
>15gm%	1	20

Chart– 6:Distribution of the cases based on the haemoglobin concentration and the severity of the disease



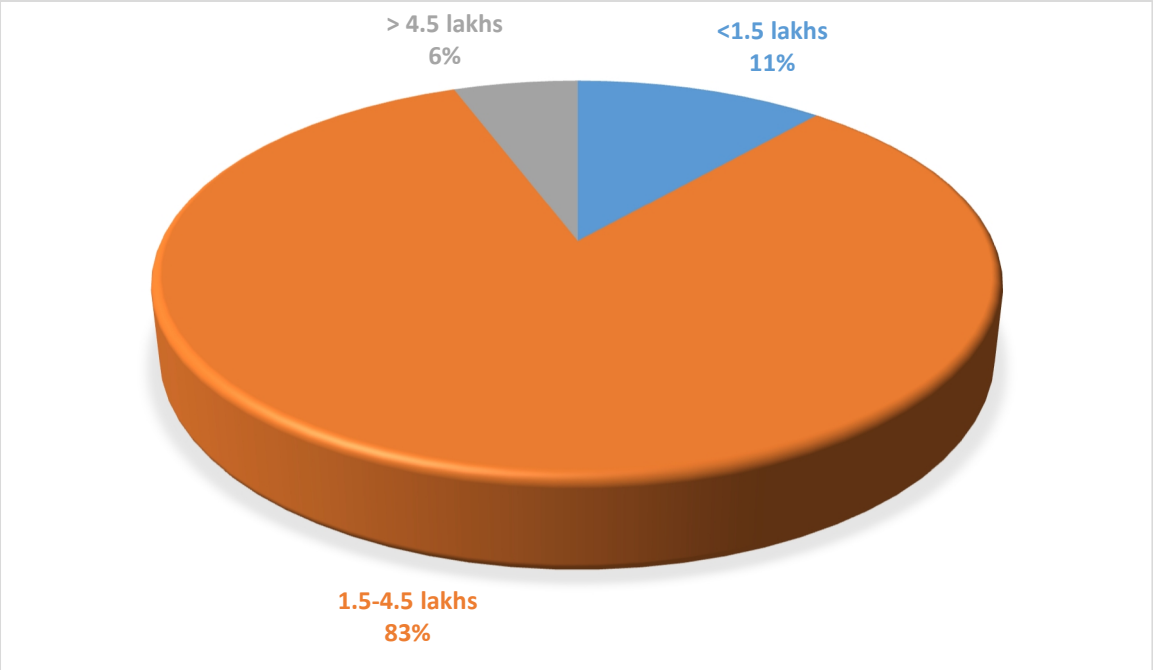
g)Distribution of the cases based on the platelet count

Out of 35 cases, 4 cases had platelet count <1.5 lakhs; 29 cases had platelet count between 1.5-4.5 lakhs; 2 cases had platelet count >4.5 lakhs. (Table – 7,Chart- 7)

Table – 7: Distribution of the cases based on the platelet count

Platelet Count	No. of cases	Percentage
<1.5 lakhs	4	11.4
1.5-4.5 lakhs	29	82.9
> 4.5 lakhs	2	5.7

Chart- 7: Distribution of the cases based on the platelet count



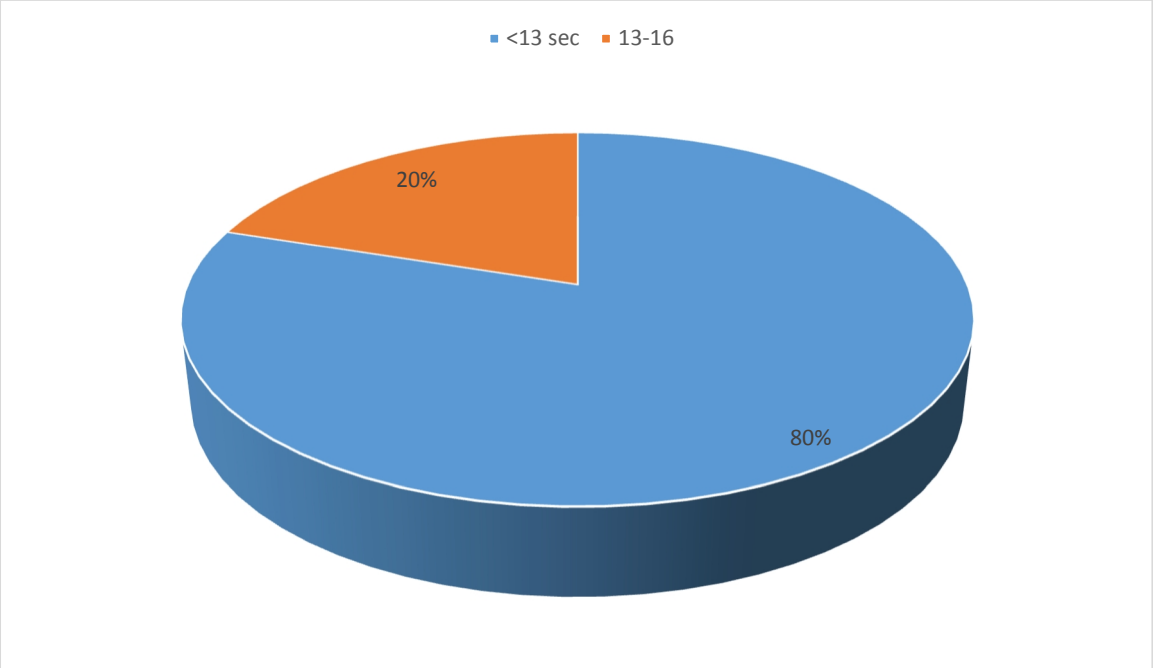
h)Distribution of cases based on the prothrombin time

Out of 35 cases all the cases had normal prothrombin time <16sec, 28 patients had prothrombin time <13 sec and 7 patients had PT between 13-16sec. (Table – 8,Chart- 8)

Table – 8: Distribution of cases based on the prothrombin time

PT Sec	No. of cases	Percentage
<13	28	80
13-16	7	20

Chart- 8: Distribution of cases based on the prothrombin time



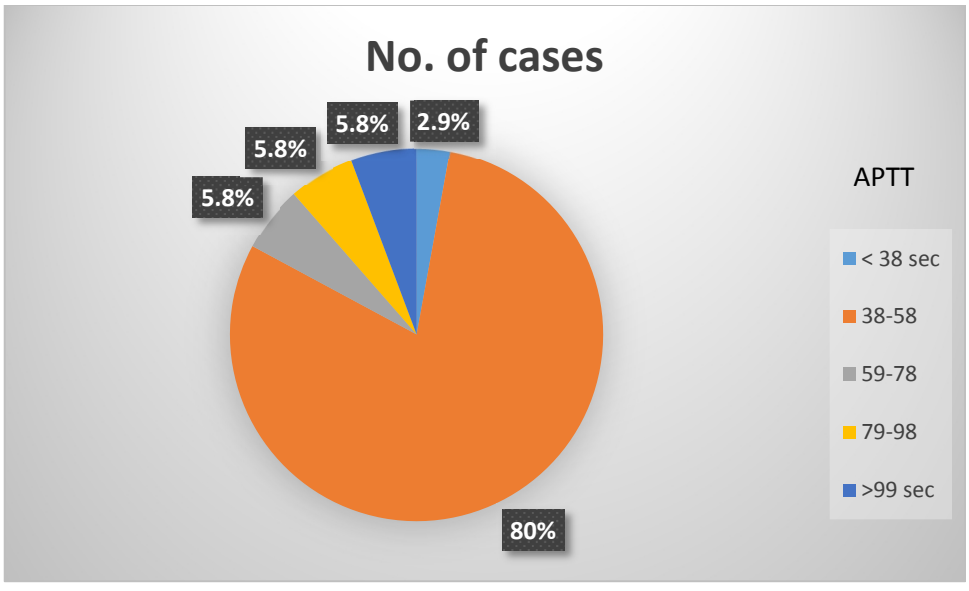
i)Distribution of cases based on the activated partial thromboplastin time

Out of 35 cases, 34 cases had elevated partial thromboplastin time, among them 28 patients had APTT between 38-58 sec, 2 patients had APTT between 59-78 sec, another 2 patients had APTT between 79-98 sec , APTT value of >99 was noted in 2 patients. 1 patient had APTT within normal range (i.e.,) below 38 sec(Table – 9,Chart- 9)

Table – 9: Distribution of cases based on the activated partial thromboplastin time

APTT (sec)	No. of cases	Percentage
< 38 sec	1	2.9%
38-58	28	80%
59-78	2	5.8%
79-98	2	5.8%
>99 sec	2	5.8%

Chart- 9: Distribution of cases based on the activated partial thromboplastin time



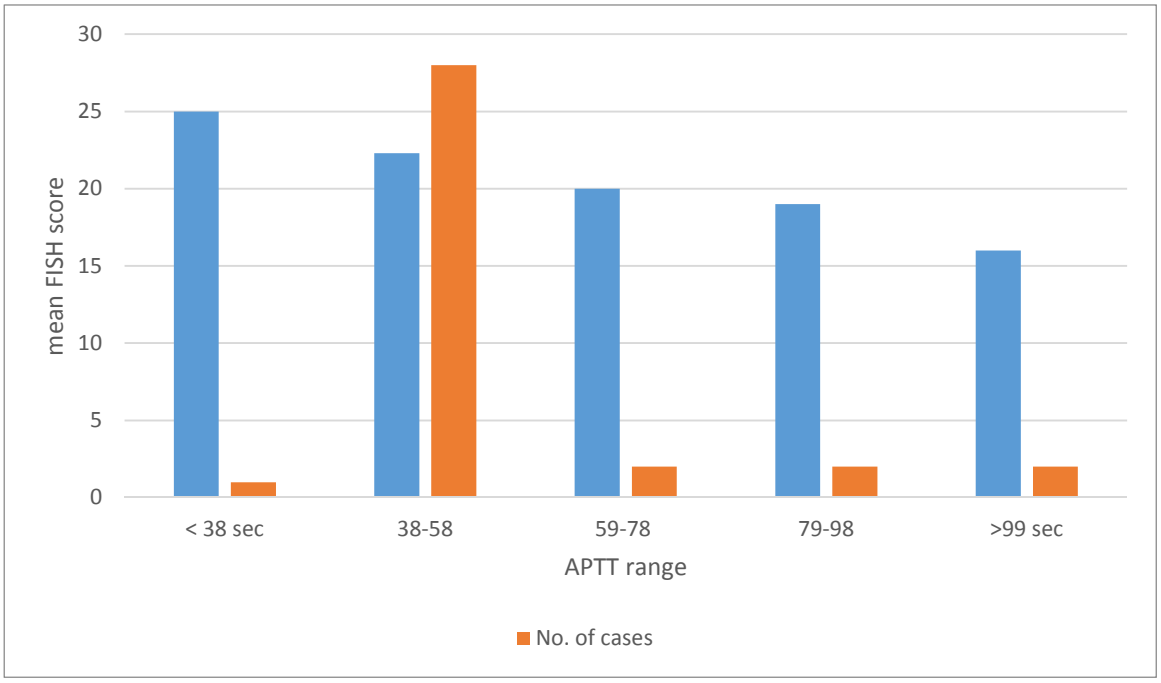
j)Distribution of cases based on the activated partial thromboplastin time and the severity of the disease

out of 35 cases, 34 cases had elevated activated partial thromboplastin time .among them 28 patients had APTT between 38-58 sec with a mean FISH score of 22, 2 patients had APTT between 59-78 sec and their mean FISH score was 20 , another 2 patients had APTT between 79-98 sec with a mean FISH score of 19 , APTT value of >99 was noted in 2 patients and they had a mean FISH score of 16. 1 patient had APTT within normal range (i.e.,) below 38 sec and his FISH score was 25(Table – 10,Chart- 10)

Table-10: Distribution of cases based on the activated partial thromboplastin time and the severity of the disease

APTT (sec)	Mean FISH score	No. of cases
< 38 sec	25	1
38-58	22.3	28
59-78	20	2
79-98	19	2
>99 sec	16	2

Chart-10: Distribution of cases based on the activated partial thromboplastin time and the severity of the disease



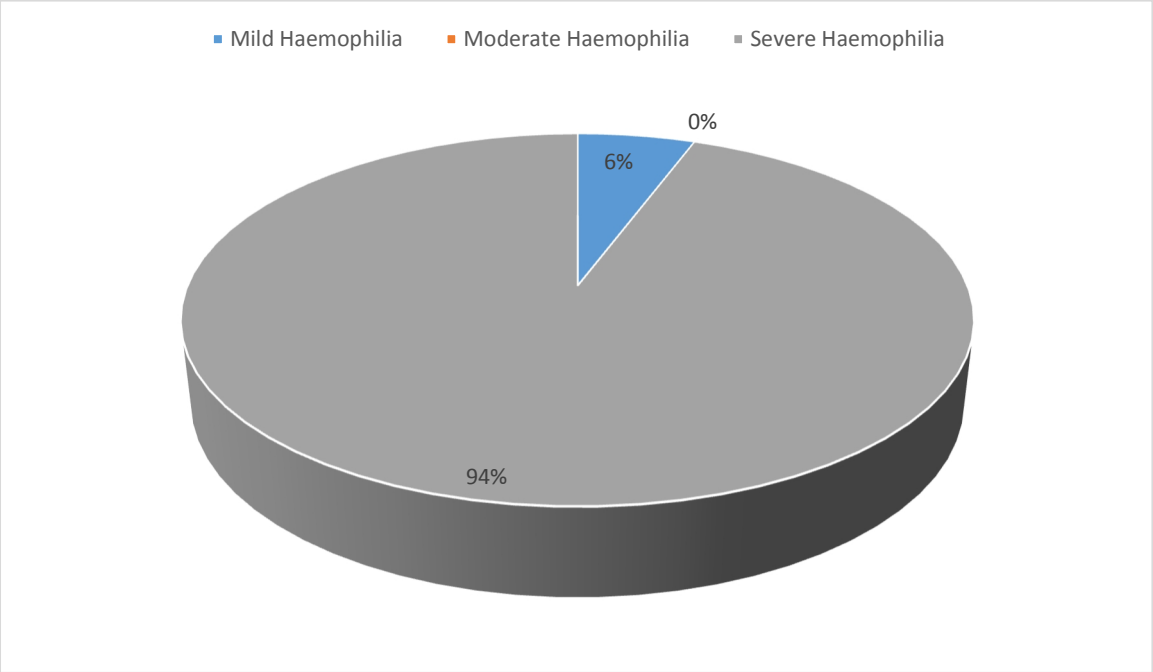
k)Distribution of cases based on F-VIII/F-IX assay

Out of 35 cases – 33 cases had F-VIII/IX activity <1% (i.e.,) severe haemophilia and 2 patients had F-VIII/IX activity >6% (i.e.,) Mild haemophilia. No cases were in the group of moderate haemophilia. (Table – 11,Chart- 11)

Table – 11: Distribution of cases based on F-VIII/F-IX assay

Type of Haemophilia	No. of cases	Percentage
Mild Haemophilia	2	5.7
Moderate Haemophilia	0	0
Severe Haemophilia	33	94.3

Chart- 11: Distribution of cases based on F-VIII/F-IX assay



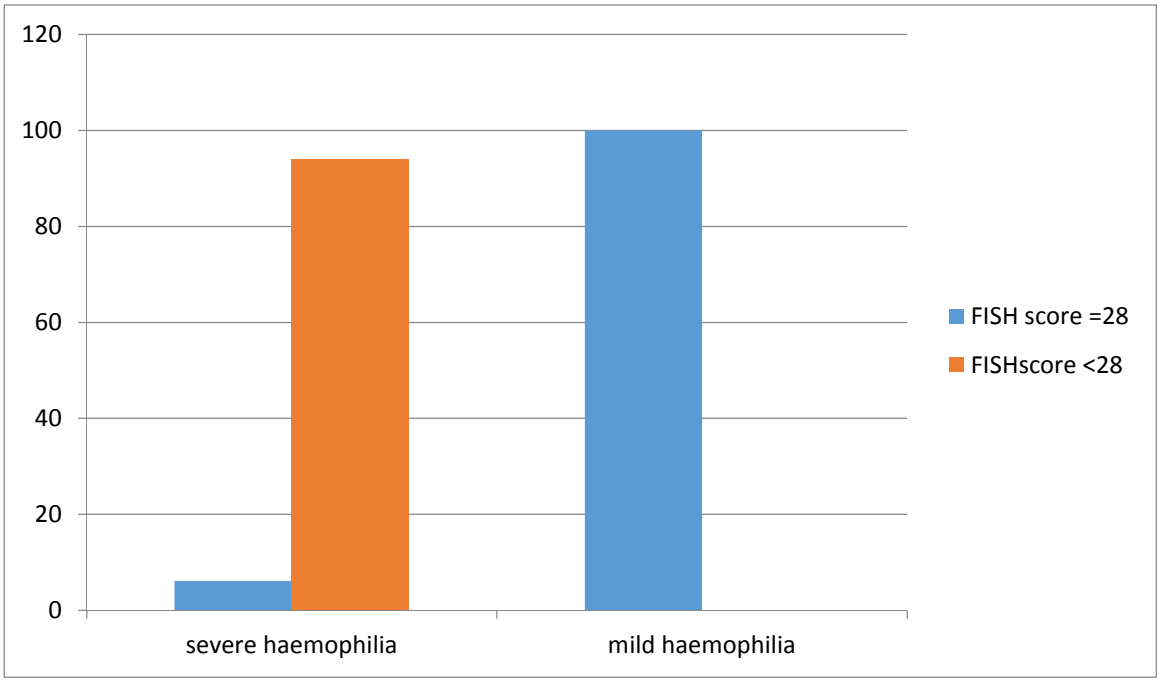
l) Distribution of the cases based on the severity of haemophilia and FISH score

Out of 35 cases 4 cases had FISH score =28. Other 28 cases had FISH SCORE ranging from 15-25 . Out of 4 cases with FISH score =28, 2 cases are mild haemophilia and 2 cases are severe haemophilia. The elder most patient in our study was 70 years and he had the lowest FISH score of 15 . (Table – 12,Chart-12)

Table – 12: Distribution of the cases based on the severity of haemophilia and FISH score

Severity of haemophilia	FISH score	
	=28	<28
Severe	2 6.1%	31 93.9%
Mild	2 100%	0 0%

Chart- 12: Distribution of the cases based on the severity of haemophilia and FISH score



Statistical analysis was done using Fisher's Exact Test and p value of 0.01 was obtained which indicates it is significant.

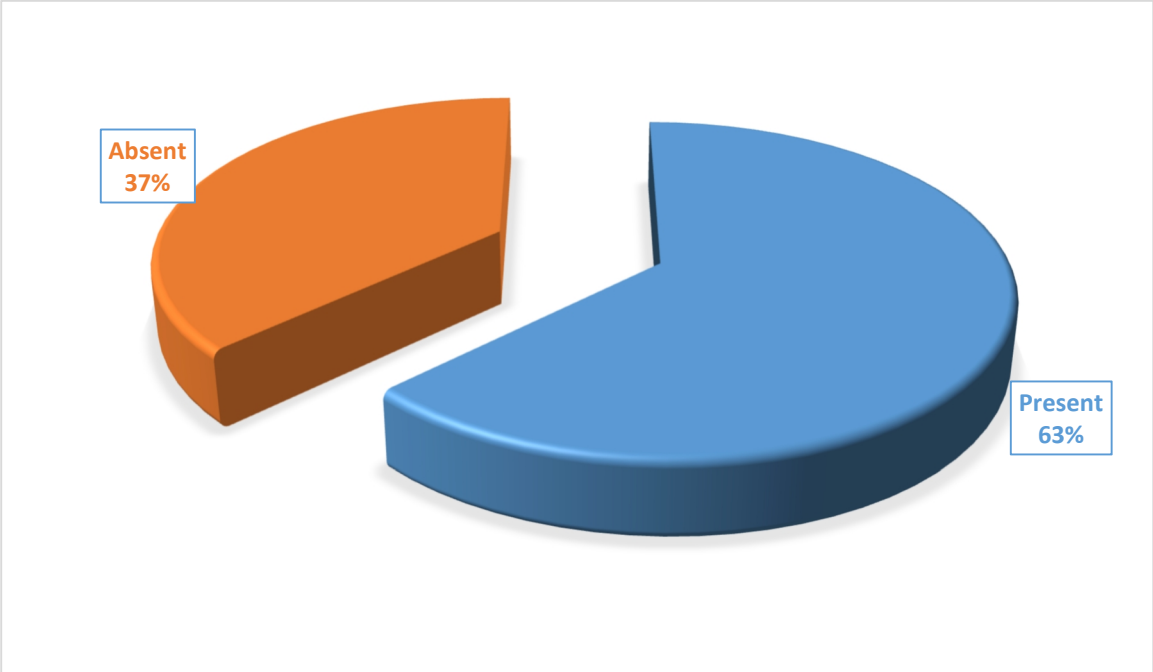
m) Distribution of cases based on the family history of haemophilia

Out of 35 haemophilia patients; 22 patients had family history of haemophilia and 13 had no family history. (Table – 13,Chart- 13)

Table – 13: Distribution of cases based on the family history of haemophilia

Family history	No. of cases	Percentage
Present	22	62.9
Absent	13	37.1

Chart- 13: Distribution of cases based on the family history of haemophilia



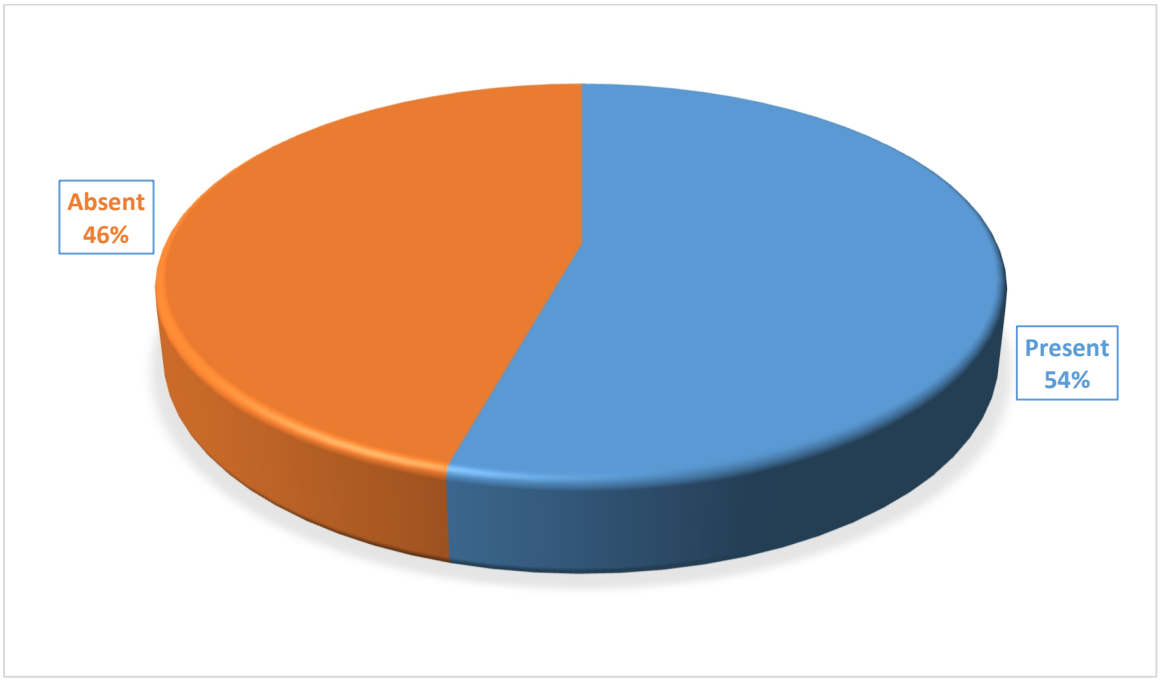
n) Cases distributed based on blood and blood product transfusion

Out of 35 total cases of haemophilia 19 patients had history of blood and blood product transfusion and16 patients had not taken any blood products. (Table – 14,Chart- 14)

Table – 14: Cases distributed based on blood and blood product transfusion

Blood & Blood Product Transfusion	No. of cases	Percentage
Present	19	54.3
Absent	16	45.7

Chart- 14: Cases distributed based on blood and blood product transfusion



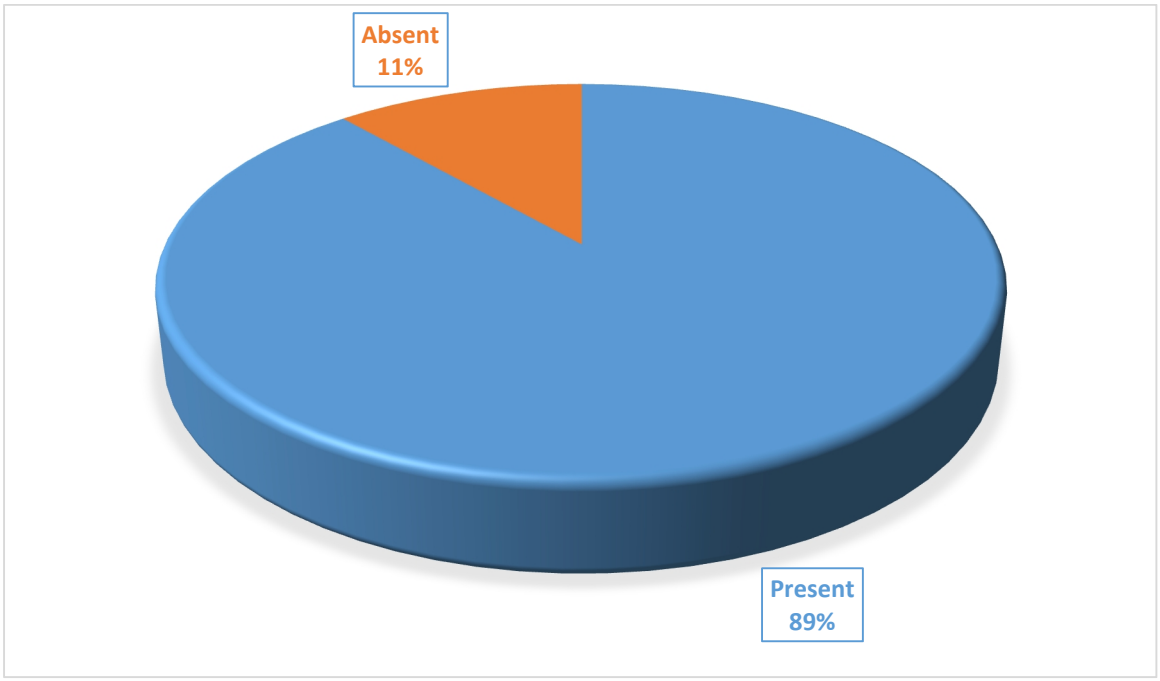
o) Distribution of cases based on joint involvement

Out of 35 cases, 31 had joint involvement by the disease, other 4 had soft tissue hematoma. (Table – 15,Chart- 15)

Table – 15: Distribution of cases based on joint involvement

Joint Involvement	No. of Cases	Percentage
Present	31	88.6
Absent	4	11.4

Chart- 15: Distribution Of Cases Based On Joint Involvement



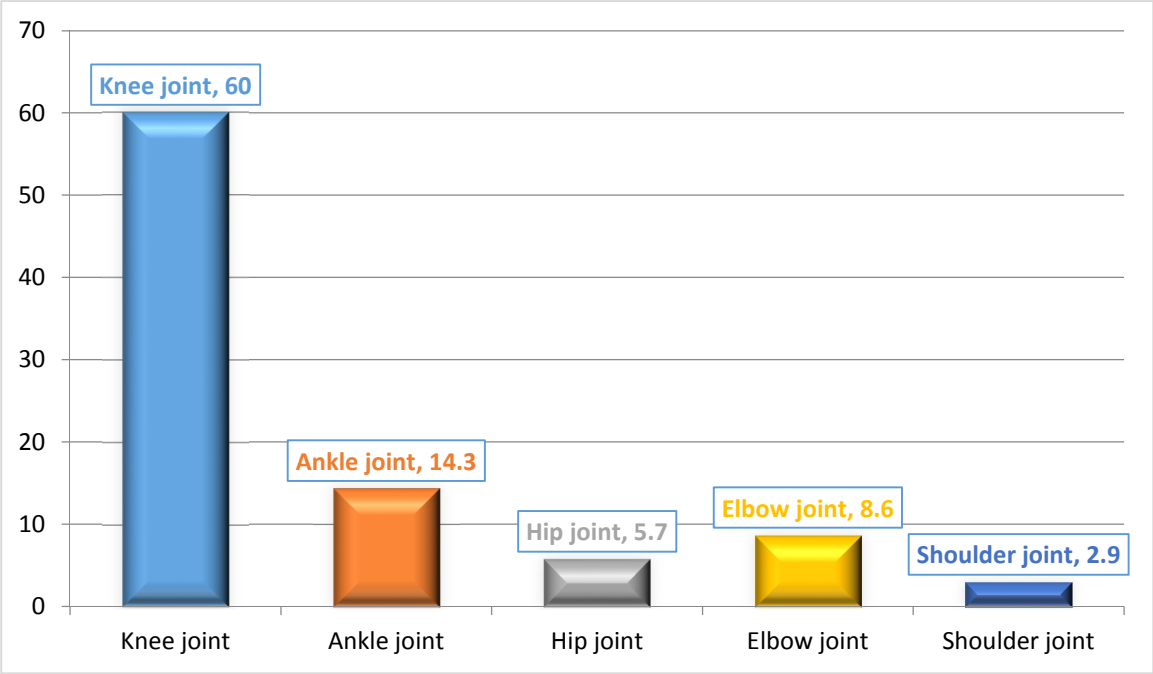
p)Incidence of cases based on the joint involved

Among 35 patients Knee joint involvement was seen in 21 patients; ankle joint involvement in 5 patients hip joint involvement in 2 patients, elbow joint involvement in 3 patients, shoulder joint involvement in 1 patient. (Table – 16,Chart- 16)

Table – 16: Incidence of cases based on the joint involved

Joint Involved	No. of Cases	Percentage
Knee joint	21	60.0
Ankle joint	5	14.3
Hip joint	2	5.7
Elbow joint	3	8.6
Shoulder joint	1	2.9

Chart- 16:Incidence of cases based on the joint involved



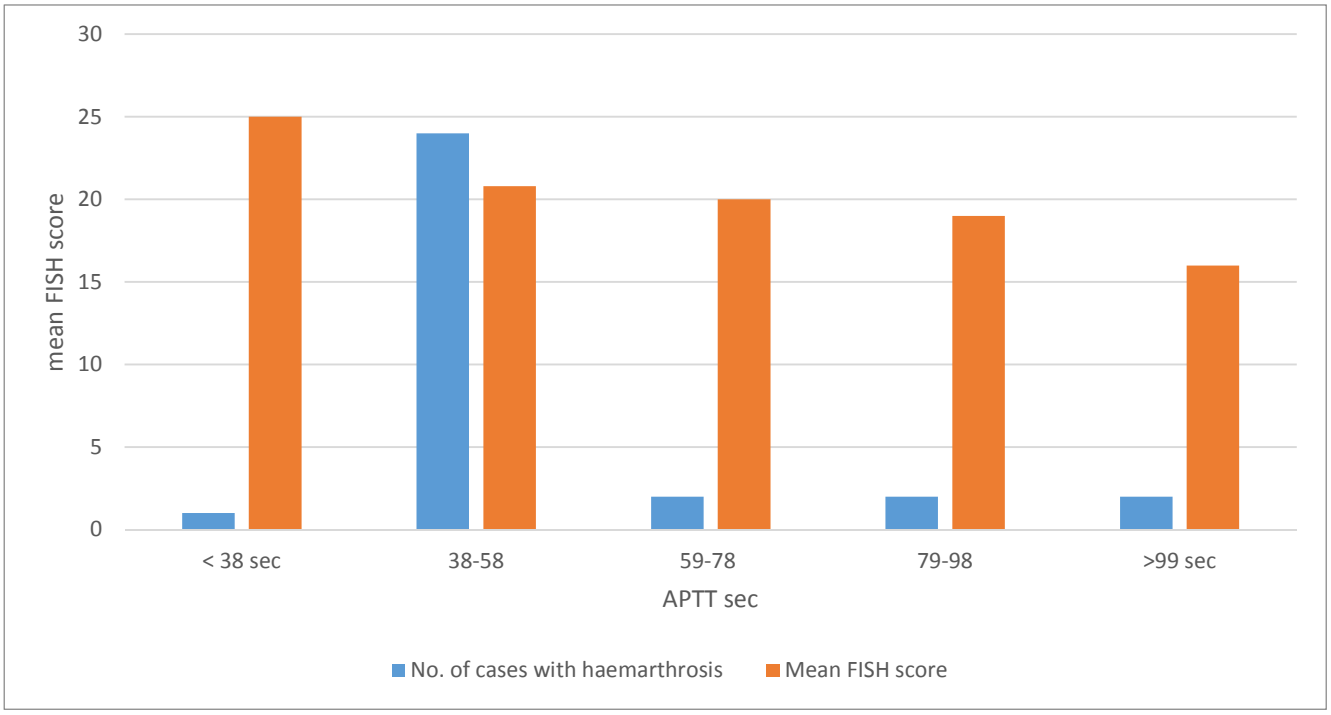
q) Comparison between APTT assay and FISH score in haemophilia patients with haemarthrosis

Out of 35 cases, 31 cases had joint involvement. Among them 1 patient had APTT <38 sec and mean FISH score of 25. Another 25 patients had APTT between 39-58 sec with mean FISH score of 20.8. Within the APTT range of 59-78 there are 2 patients with a mean FISH score of 20. 2 patients had APTT between 79-98 and they had a mean FISH score of 19. Other 2 patients had APTT >99 sec mean FISH score of 16.(Table -17,Chart- 17)

Table – 17: Comparison between APTT assay and FISH score in haemophilia patients with haemarthrosis

APTT (sec)	No. of cases with haemarthrosis	Mean FISH score
< 38 sec	1	25
38-58	24	20.8
59-78	2	20
79-98	2	19
>99 sec	2	16

Chart– 17: Comparison between APTT assay and FISH score in haemophilia patients with haemarthrosis



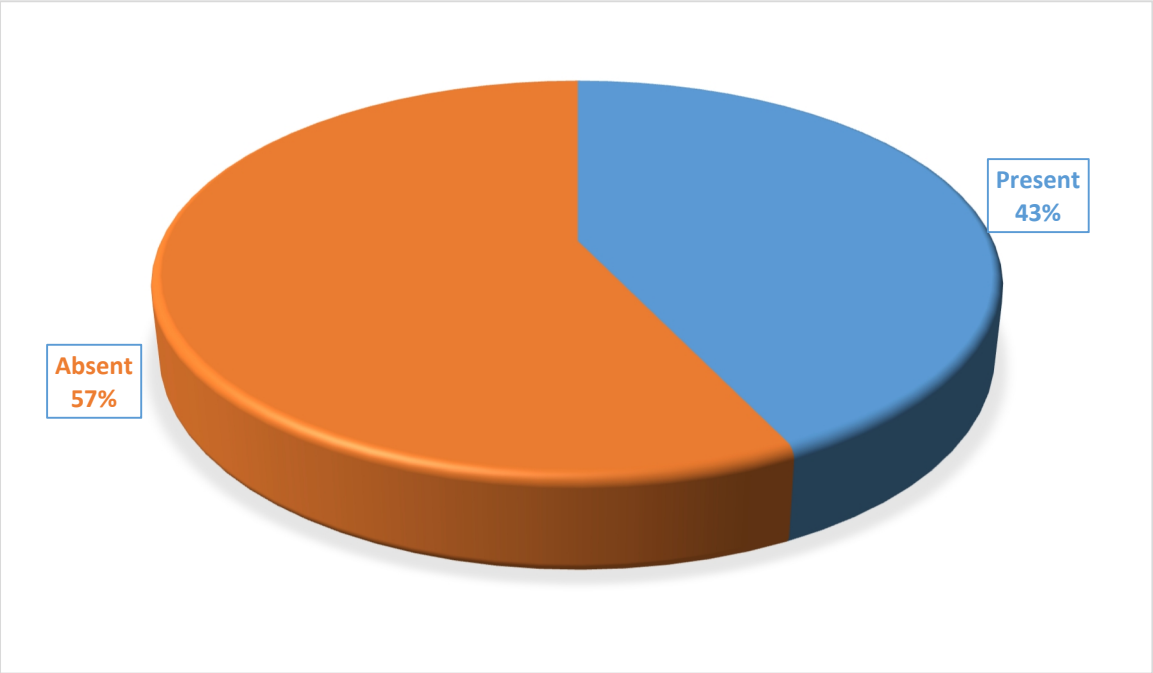
r)Distribution of cases based on soft tissue bleeding

Out of 35 cases 15 had history of soft tissue bleeding (Table -18,Chart- 18)

Table – 18: Distribution of cases based on soft tissue bleeding

Soft Tissue bleeding	No. of cases	Percentage
Present	15	42.9
Absent	20	57.1

Chart- 18: Distribution of cases based on soft tissue bleeding



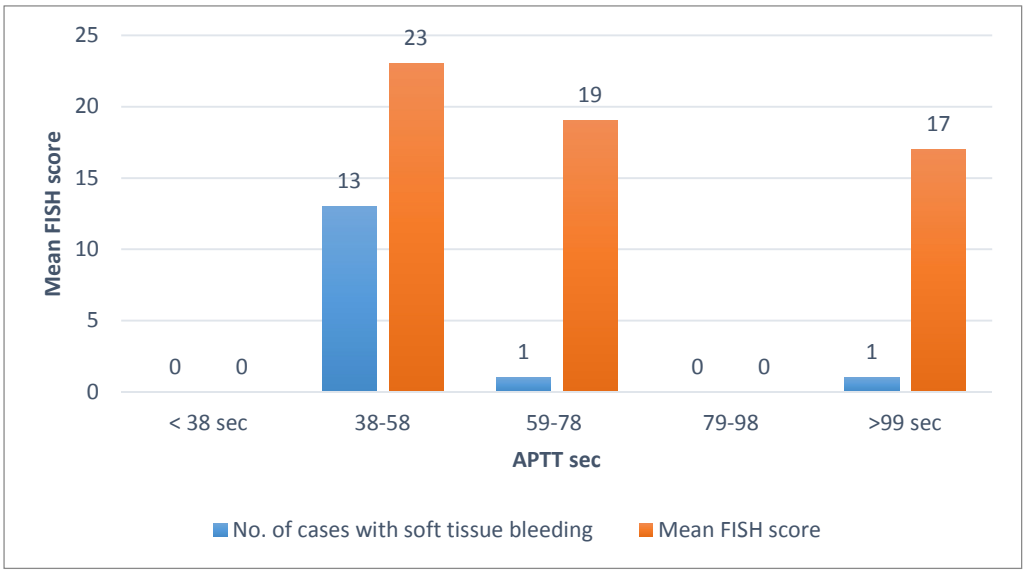
s) Comparison between APTT assay and FISH score in haemophilia patients with soft tissue bleeding

Out of 35 cases, 15 cases had soft tissue bleeding. Among them 13 patients had APTT between 39-58 sec and mean FISH score of 23. Another 1 patient had APTT between 59-78 sec with mean FISH score of 19. One patients had APTT >99 sec mean FISH score of 17.(Table -18,Chart- 18)

Table – 18:Comparison between APTT assay and FISH score in haemophilia patients with soft tissue bleeding

APTT (sec)	No. of cases with soft tissue bleeding	Mean FISH score
< 38 sec	0	-
38-58	13	23
59-78	1	19
79-98	0	-
>99 sec	1	17

Chart – 18:Comparison between APTT assay and FISH score in haemophilia patients with soft tissue bleeding



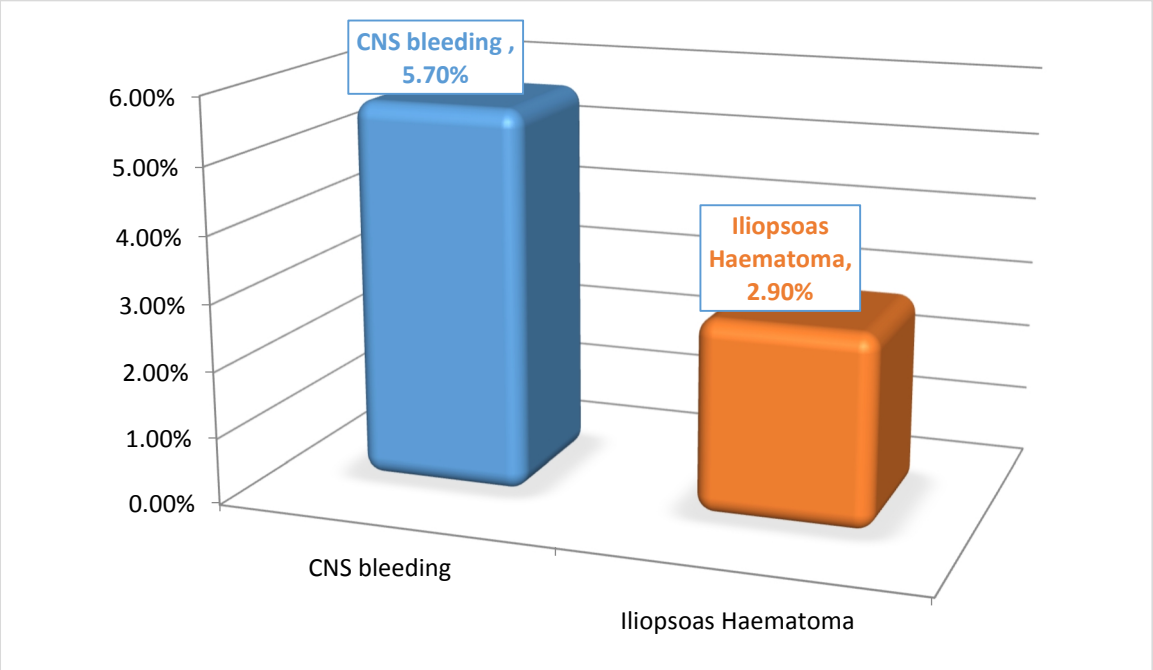
t)Distribution of cases based on life threatening bleeding manifestations

Out of 35 cases; 2 cases had history of CNS bleeding and 1 case had history of Iliopsoas Haematoma formation. (Table – 19,Chart- 19)

Table – 19: Distribution of cases based on life threatening bleeding manifestations

Life Threatening Haematoma	No. of cases	Percentage
CNS bleeding	2	5.7
Iliopsoas Haematoma	1	2.9

Chart- 19: Distribution of cases based on life threatening bleeding manifestations



t)Distribution of cases based on the number of admissions in the day care centre

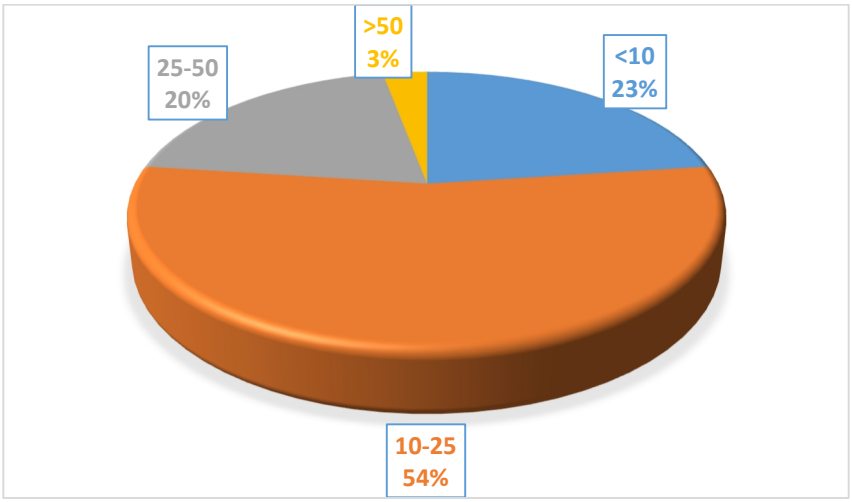
out of 35 cases, 8 patients had <10 admissions during the study period, 19 patients had 10-25 admissions , 7 patients had 25-30 admissions ,1 patient had >50 admissions during the study period.

(Table – 20,Chart- 20)

Table – 20: Distribution of cases based on the number of admissions in the day care centre

No. of admission	No. of Cases	Percentage
<10	8	22.9
10-25	19	54.2
25-50	7	20
>50	1	2.9

Chart- 20: Distribution of cases based on the number of admissions in the day care centre



DISCUSSION

Haemophilia is a common hereditary bleeding disorder . The etiology of haemophilia is the deficiency of coagulation factor VIII and IX . Disruption in the coagulation cascade leads to impaired production of fibrin clot . Depending on the level of clotting factors, haemophilia is divided into mild , moderate and severe forms.

Severe haemophilia is associated with < 1 % of factor level and is characterized by development of recurrent joint bleeding and chronic arthropathy. A performance based assessment tool is used to measure the functional ability of patients called as functional independence score in haemophilia. The patients with haemophilia A and B are treated with intravenous infusion of factor VIII and factor IX respectively . Dosage depends on the severity of the symptom complex . Few of these patients are positive for inhibitors and they are treated with Inj. Nova Seven which is recombinant activated factor VII (rFVIIa).

Age

The age of diagnosis is < 5 years in majority of patients with maximum cases diagnosed during the first two years of life.

The age of diagnosis of haemophilia ranges from at birth in patients with family history of haemophilia to 2 years . Higher frequency of diagnosis is

between 1.2 – 2 years of age , during which the child starts walking and there is increased risk of bleeding into joints .

In our study, 28 cases out of 35 i.e 80% are diagnosed before the age of 5 years. In studies by Sonata Saulyte Trakymiene et.al.,[145]most cases are diagnosed at the first episode of joint bleed which occurs between 1-5 years of age with a maximum frequency of cases occurring between 1.2-2 years of age. In a study by Jamie O'Hara et.al.,[83]it was said that in the absence of preventive 'prophylaxis' factor replacement therapy, patients with severe haemophilia (<1% of normal factor level) will develop first episode of haemarthrosis between the ages of 1 and 5 years.

Prevalence

In our study haemophilia A is more common than haemophilia B . Among 35 cases under study, 30 cases are haemophilia A accounting for 87.7 % and 5 cases are haemophilia B accounting for 12.3 %. The observations published by World Federation of Haemophilia also states that haemophilia A , is more common than haemophilia B representing 80 – 85 % of total haemophiliacs population . In studies by Hassan mansouritorghabeh et.al., [90]it is stated that Haemophilia A or classical hemophilia accounts for about 80% of all hemophiliacs.

In our study , out of 35 cases under study 4 cases are positive for inhibitors and they are treated with Inj . Nova Seven . They account for 14.7 % of study population . Mean age of patients positive for inhibitors is 18.75years.

According to a study on inhibitor development in children by Samantha Claudia Gowl et . al ,[135] the development of inhibitors occurs in around 20 – 30 % patients with haemophilia A . A study by Karin Knobe et.al.,[75] stated that development of inhibitors is around 20% in patients with haemophilia. Studies by Wight J, Paisley S. et.al.,[146] and other study by Gouw SC, van den Berg HM, Fischer K et al.,[129] also states that the incidence of development of inhibitors is around 30%.

In our study among 4 inhibitor positive cases two patients had APTT of 42.1 and 39.5 (range 39-58 sec) with a FISH score of 25. one patient had APTT value of 40.2 (range 39-58 sec) and FISH score of 20, another one patient had APTT value of 92.7 (range 79-98sec) and FISH score of 20. From this it was noted that inhibitor positive patient with elevated APTT had lowest FISH score indicating severe disability. These patients had more than one joint involvement and soft tissue hematomas.

Platelet Count

In our study platelet count is < 1.5 lakhs in 4 out of 35 cases (1.4 %) and platelet count is in the normal range in 29 cases out of 35 (82.9 %) and 2 cases

out of 35 (5.7 %) have platelet count >4.5 lakhs. This correlated well with study done by Agarwal et al.[147] and T. B. Sadaria et.al.,[148] which showed that platelet count of all patients was ranging from 1,50,000 to 4,91,000 /cmm.

Haemoglobin

In our study out of 35 cases, 3 cases had haemoglobin in the range of 7-10 gms% and they had a mean FISH score of 21; 31 cases were in the haemoglobin range of 10-15 gms% also had a mean FISH score of 21; 1 case with haemoglobin >15 gms% had a mean FISH score of 20.

In studies by Sagir G.ahmed et.al[157] it was stated that patients with haemophilia are more prone for iron deficiency and presents with iron deficiency anaemia. A study by Paranthaman poongavanam et.al[158] showed that iron deficiency is common in patients with haemophilia and it is related with increase severity of the disease due to repeated episodes of spontaneous bleed. In our study haemoglobin values doesnot correlate with the severity of the disease as patients with anaemia and haemoglobin within normal range had a mean FISH score of 21 and a patient with above normal haemoglobin had a low FISH score of 20. Patients with normal haemoglobin range also had severe clinical manifestations in the form of haemarthrosis involving two or more joints and soft tissue hematomas.

Prothrombin time

Prothrombin time of all 35 patients was within normal limits<16 sec . This correlated well with study done by Kitchens CS.et.al.,[150]and T. B. Sadaria et.al.,[148] which stated that PT is normal in all patients with haemophilia as extrinsic pathway is intact in haemophilia.

Activated partial thromboplastin time

APTT was prolonged in 34 cases out of 35 cases which accounts for 97.1% and in 1 case out of 35 cases APTT was within the normal range. Among them 28 patients had APTT between 38-58 sec, 2 patients had APTT between 59-78 sec, another 2 patients had APTT between 79-98 sec , APTT value of >99 was noted in 2 patients.It is the most efficient screening test available to suspect a case of haemophilia and this correlated well with the severity of the disease and severity of disease manifestations . In a study done by Kitchens CS.et.al.,[150] and Takim &Shrivastava et.al[151]. it was found that APTT was prolonged in all the patients with haemophilia as it involves disruption of the intrinsic pathway of coagulation.

Elevated APTT values were correlated with the severity of the disease in terms of mean FISH score. In that out of 35 cases, 34 cases had elevated activated partial thromboplastin time .among them 28 patients had APTT between 38-58 sec with a mean FISH score of 22, 2 patients had APTT between 59-78 sec and

their mean FISH score was 20 , another 2 patients had APTT between 79-98 sec with a mean FISH score of 19 , APTT value of >99 was noted in 2 patients and they had a mean FISH score of 16. 1 patient had APTT within normal range (i.e.,) below 38 sec and his FISH score was 25. It was noted that for progressive increase in APTT values the mean FISH score was decreasing indicating that increase in APTT is associated with the increasing severity of the disease.

Functional Independence Score in Hemophilia (FISH)

FISH is a performance based assessment tool which measures the functional ability of patients with haemophilia . FISH score analysis shows that the lowest scores are for squatting. Squatting was the most difficult task and these patients must be given early toilet training to use western style lavatories as this may avoid stress on knee joint and delay the onset of joint arthropathy . According to a study on FISH and factors affecting it by Dr Malathi et . al , [2]it was said that the FISH score was low for squatting and this correlates with our study . This study also shows that as the age advances FISH score becomes lower and this correlates well with our study . The oldest patient in our study was 70 years and he had the lowest FISH score of 15 .

Most commonly involved joint in our study is the knee joint . In 21 cases out of 35 cases knee joint was involved accounting for 60 % , followed by ankle joint , in 5 cases out of 35 cases accounting for 14.3 % .This result correlated with the study by Dr Malathi et . al [2]; which showed that knee joint was the most

commonly involved joint as it lacks adequate muscle cover and it is not able to withstand rotator and angular stress .

In our study FISH score was low in patients with severe haemophilia compared to mild haemophilia cases who had little or no functional impairment .

FISH score for severe haemophilia patients was found to be <28 and the lowest value was found to be 15. In mild haemophilia patients FISH score was normal and it was found to be equal to 28. Statistical analysis was done using Fischer's Exact test and p value < 0.01 is obtained. It is statistically significant . This finding correlated with the study done by Alberto Tlacauto Parra et . al .,[152] which show no functional changes in patients with mild haemophilia and functional impairment in patients with severe haemophilia.

In our study Knee joint involvement was seen in 21 patients(60%); ankle joint involvement in 5 patients(14.3%) hip joint involvement in 2 patients(5.7%), elbow joint involvement in 3 patients(8.6%), shoulder joint involvement in 1(2.9%) patient.Studies by Johannes Oldenburg [113]states that for patients not on prophylaxis knee joint bleeds are the most frequently affected joints. Other studies by Karin Knobe et.al.,[75] states that the in patients on on-demand therapy the joints are affected in the frequency of knees (45%), followed by the elbows (30%), ankles (15%), shoulders (3%), and wrists (2%).

This correlates with our study where knee joint is the most frequently affected joint.

31 out of 35 cases with joint involvement were compared by elevated APTT and mean FISH score. Among them 1 patient had APTT <38 sec and mean FISH score of 25. Another 25 patients had APTT between 39-58 sec with mean FISH score of 20.8. Within the APTT range of 59-78 there are 2 patients with a mean FISH score of 20. 2 patients had APTT between 79-98 and they had a mean FISH score of 19. Other 2 patients had APTT >99 sec mean FISH score of 16. It was found that patients with increased APTT had increased frequency of haemarthrosis and increased severity of the disease as mean FISH score is progressively decreasing in patients with elevated APTT.

From 15 cases of soft tissue bleeding, 13 patients had APTT between 39-58 sec and mean FISH score of 23, 1 patient had APTT between 59-78 sec with mean FISH score of 19, another 1 patient had APTT >99 sec and his mean FISH score of 17. This indicates that prolongation of APTT correlates with the severity of the disease. APTT assessment can act as a screening test and the degree of increase in APTT can be used as a valuable indicator of the disease severity in terms of functional impairment.

Life threatening haemorrhages

In our study out of 35 cases; 2 cases had history of CNS bleeding accounting for 5.7% and 1 case had history of Iliopsoas haematoma formation accounting for 2.9%.In studies by I. E. Morsing et.al.,[86] it was stated that intracranial hemorrhageafter the neonatal period occured in 3 – 10 % of the patients.

Another study by Ajay Hegdeet.al.,[155] stated that spontaneous intracerebral haemorrhage is a rare complication of hemophilia occurring at a frequency of about 2.2–7.8%.A study done by Marc Dauty et,al.,[156] states that the incidence of iliopsoas hematoma in patients with severe haemophilia was 2.9/1000patients with severe or moderate haemophilia.

Inheritance

In our study 22 cases out of 35 (i.e) 62.9 % had come from " affected families " i.e those with haemophilia prevalent in their previous generations . They had siblings or maternal uncle suffering from the disease . In our study 13 cases out of 35 (i.e) 37.1% % were the first in their families to be affected by haemophilia. This correlates with the study done by Kate Khair et . al [153]and Anita Kar et.al[154] which stated that around 30% of cases arise due to spontaneous mutations without any previous family history.

In the present study haemoglobin concentration of the patients were analysed and it was found that patients with haemoglobin <10 gm% and haemoglobin between 10-15 gm% had FISH score of 21 and a patient with haemoglobin >15 gm% also had low FISH score of 20. So, in our study it was found that haemoglobin concentration doesnot correlate with the disease severity.

In our study APTT values of the haemophilia patients were analysed and compared with the severity of the disease. Among 35 patients 34 patients show

elevated APTT . This also includes 2 mild haemophilia patients with APTT of 39.2 and 40.3 with a mean FISH score of 28. Mild haemophilia patients also showed elevation of APTT[159]. They presented with symptoms like soft tissue hematomas and prolonged bleeding after trauma. Their FISH score was 28 and they were able to perform all the day to day activities.

In other 33 patients with severe haemophilia 32 patients show elevated APTT ranging from >38 sec to 108sec. Mean FISH score of these patients was 19 indicating disability. Presentations in these patients ranges from soft tissue bleeds, prolonged bleeding after trauma and spontaneous haemarthrosis. This showed that in patients with severe haemophilia as symptoms increases, indicated by decreasing FISH score APTT values are increased. So, APTT can be used as a screening tool to determine the severity of the disease and time of administration of factor concentrates in patients with haemophilia.

In our study among 4 inhibitor positive haemophilia patients with elevated APTT , one patient had APTT value of 92sec and lowest FISH score of 20. This indicates that in inhibitor positive patients APTT assay can be used to identify the severity of the bleeding episode and the time interval within which factor concentrates to be infused.

SUMMARY AND CONCLUSION

In our study , haemophilia patients admitted to the day care centre of Tirunelveli medical college hospital are analysed. It was found that haemophilia is a rare inherited X-linked recessive bleeding disorder. The bleeding manifestation in these patients varies from lethal CNS hemorrhagic episodes to superficial ecchymosis. Performing a complete physical examination, gathering the entire past medical history of the cases and the family plays an important role in the study of the disease.

Basic laboratory parameters like CBC and coagulation profile were analysed. Haemoglobin estimation showed that the degree of anaemia or increased haemoglobin concentration in haemophilia patients under study had no correlation with the severity of the disease.

APTT values in haemophilia patients correlated with the severity of symptoms. Patients with increased APTT had lowest FISH score and severe symptoms like multiple joint involvement leading to multiple joint haemarthrosis with associated soft tissue hematomas. This can act as a key to estimate the severity of the disease and the time by which factor replacement therapy has to be given.

Inhibitor positive haemophilia patients also had elevated APTT and the degree of elevation correlated with the severity of the disease. So, it is clear that APTT can be used as a guiding tool to help the treating physician in determining the time by which factor concentrates have to be given. It gives an

idea whether factor replacement should be made immediately or patient can wait for some time without any complications for factor concentrates.

Though FISH score has been a vital tool in assessing the severity of the disease, haemoglobin and APTT could help in effective management of haemophilic patients on regular therapy.

BIBILIOGRAPHY

1. Massimo Franchini1 and Pier Mannuccio Mannucci. Past, present and future of hemophilia: a narrative reviewFranchini and Mannucci Orphanet Journal of Rare Diseases 2012, 7:24 http://www.ojrd.com/content/7/1/24
2. Dr. S. Malathi Dr. S. Kalaichelvi Dr. Sivanesan Dr. R. Vijay Usha Raj Dr. V. Madhavan. Functional Independence Score in Hemophiliacs and Factors affecting it. DOI: 10.21276/sjams.2016.4.6.67
3. Stonebraker JS, Bolton-Maggs PH, Soucie JM,Walker I, Brooker M. A study of variations in thereported haemophilia A prevalence around the world.Haemophilia 2010;16(1):20-32.
4. Anwarul Karim, Chowdhury Yakub Jamaa Review On Hemophilia In Children bangladesh J Child Health 2013; Vol 37 (1) : 27-40
5. Sona P.S, C Muthu Lingam Hemophilia - An Overview. 04-11-2010.
6. Schulman S. Mild hemophilia. In: Schulman S, editor. Treatment of Hemophilia Monographs No. 41, December 2006. Montreal: World Federation of Hemophilia; 2006. Available from: http://www.wfh. org/2/docs/Publications/Diagnosis_and_Treatment/TOH-41_Mild-Hemophilia.pdf
7. Miguel A. Escobar And Nigel S Williams Hematology ninth Edition chapter 123 Hemophilia A And Hemophilia B . Isbn: 978-0-07-183300-4
8. Abdul Nasser Kaadan MD, PhD, Mahmud Angrini MD Who Discovered Hemophilia? October 2010.
9. ABU'L-QASIM ALZAHRAWI, 2004- Kitab al-Tasrif. Edited by Sobhi Mahmoud Hemmami, 1st edition
10. Rosner F: Haemophilia in the Talmud and Rabbinic writings. Ann Intern Med 1969;70:833-837.
11. Otto J.C: An account of a hemorrhagic disposition existing in certain families. Med Repos 1803;6:1-4.
12. Rosendaal FR, Smit C, Briët E: Hemophilia treatment in historical perspective: a review of medical and social developments. Ann H
13. Patek AJ, Taylor FH: Hemophilia. II. Some properties of a substance obtained from normal human plasma effective in accelerating the coagulation of hemophilic blood. J Clin Invest 16:113–124, 1937.
14. Giangrande PLF: Six characters in search of an author: The history of the nomenclature of coagulation factors. Br J Haematol 121:703–712, 2003.
15. Denson K: Electrophoretic studies of the Prower factor: A blood coagulation factor which differs from factor VII. Br J Haematol 4:313–325, 1957.
16. Aggeler PM, White SG, Glendening MB, et al: Plasma thromboplastin component (PTC) deficiency; a new disease resembling hemophilia. Proc Soc Exp Biol Med 79:692–694, 1952.

17. Ratnoff O, Colopy J: A familial hemorrhagic trait associated with a deficiency of a clot-promoting fraction of plasma. J Clin Invest 34:602–613, 1955.
18. Marder VJ, Budzynski AZ: The structure of the fibrinogen degradation products. ProgHemost Thromb 2:141–174, 1974.
19. Kunicki TJ, Baronciani L, Canciani MT, et al. An association of candidate gene haplotypes and bleeding severity in von Willebrand Disease (VWD) type 2A, 2B, and 2M pedigrees. J Thromb Haemost 2006;4:137-147.
20. Mettine H. A. Bos, Cornelis Van 'T Veer, And Pieter H. Reitsma Molecular Biology And Biochemistry Of The Coagulation Factors And Pathways Of Hemostasis Williams HematologyNinth EditionCHAPTER 113. page.1925.
21. Biggs R, Douglas AS, MacFarlane RG, et al: Christmas disease: A condition previously mistaken for haemophilia. *Br Med J* 2:1378–1382, 1952.
22. Briet **E,** Merk MD, Wijnands MD, Veltkamp J: The prophylactic treatment of hemophilia B Leyden with anabolic steroids. J Ann Intern Med 103:225, 1985
23. Bnet E, Bertina RM, van Tilburg NH, Veltkamp J: Hemophilia B Leyden: A sex-linked hereditary disorder that improves after puberty. N Engl **J** Med **306:788,** 1982
24. Giannelli F. Green PM, High KA, Sommer **S,** Lillicrap DP, Ludwig M, Olek K. Reitsma PH, Goossens M, Yoshioka **A,** Brownlee GG: Haemophilia B: Database of point mutations and short additions and deletions-third edition. Nucleic Acids Res 20:2027,1992
25. Pal Andre` Holme and Geir Erland Tjønnfjord Management of Acquired Hemophilia: A Literature Review Norway JCD 2009; 000:(000). Month 2009
26. Stephanie A. Smith, Richard J. Travers, and James H. Morrissey How it all starts: initiation of the clotting cascade Crit Rev Biochem Mol Biol. 2015 ; 50(4): 326–336. doi:10.3109/10409238.2015.1050550.
27. Alok Srivatasva, editor. Guidelines for management of hemophilia in India. Hemophilia, Hemophilia federation (India), 2003
28. Coller B: Platelets in cardiovascular thrombosis and thrombolysis, in The Heart and Cardiovascular System, edited by HA Fozzard, AM Katz, HE Morgan, E Haber: pp 219–273. Raven Press, New York, 1991.
29. Mariasanta Napolitano, Alvin H. Schmaier, Craig M. Kessler Henry's Clinical Diagnosis And Management Isbn: 978-0-323-29568-0 Laboratory Methods, 23e Chapter 39 Coagulation And Fibrinolysis .
30. Amy D. Shapiro, MD, Medical Director, and Whitney Sealls, PhD Robbins Basic Pathology tenth edition Hemophilia B. c h a p t e r 4 Hemodynamic Disorders, Thromboembolism, And Shock

31. Stephanie A. Smith, Richard J. Travers, and James H. Morrissey How it all starts: initiation of the clotting cascade
32. Biochem Mol Biol. 2015 ; 50(4): 326–336. doi:10.3109/10409238.2015.1050550.
33. Alisa S. Wolberg and Robert A. Campbell Thrombin Generation, Fibrin Clot Formation and Hemostasis .Transfus Apher Sci. 2008 February ; 38(1): 15–23. doi:10.1016/j.transci.2007.12.005.
34. Komaromi I, Bagoly Z, Muszbek L: Factor XIII: Novel structural and functional aspects.J Thromb Haemost 9:9–20,2011.
35. Silverberg M, Dunn JT, Garen L, Kaplan AP. Autoactivation of human Hageman factor. Demonstration utilizing a synthetic substrate. J Biol Chem. 1980; 255:7281–7286. [PubMed: 7391081]
36. Müller F, Gailani D, Renné T. Factor XI and XII as antithrombotic targets. Curr Opin Hematol. 2011; 18:349–355. [PubMed: 21730835]
37. Renné, T. The factor XII-driven plasma contact system. In: Marder, VJ.; Aird, WC.; Bennett, JS.; Schulman, S.; White, GC., 2nd, editors. Hemostasis and Thrombosis: Basic Principles and Clinical Practice. 6th. Philadelphia: Lippincott Williams & Wilkins; 2013.
38. Collen D, Tytgat GN, Claeys H, Piessens R: Metabolism and distribution of fibrinogen.I. Fibrinogen turnover in physiological conditions in humans. Br J Haematol 22:681– 700, 1972.
39. Blomback B: Studies on fibrinogen: Its purification and conversion into fibrin. Acta Physiol Scand Suppl 43:1–51, 1958.
40. Marder VJ, Budzynski AZ: The structure of the fibrinogen degradation products. Prog Hemost Thromb 2:141–174, 1974.
41. Royle NJ, Irwin DM, Koschinsky ML, et al: Human genes encoding prothrombin and ceruloplasmin map to 11p11-q12 and 3q21–24, respectively. Somat Cell Mol Genet 13:285–292, 1987.
42. Nesheim ME, Nichols WL, Cole TL, et al: Isolation and study of an acquired inhibitor of human coagulation factor V. J Clin Invest 77:405–415, 1986.
43. Owren PA: Parahaemophilia; haemorrhagic diathesis due to absence of a previously unknown clotting factor. Lancet 1:446–448, 1947.
44. Giangrande PLF: Six characters in search of an author: The history of the nomenclature of coagulation factors. Br J Haematol 121:703–712, 2003.
45. Camerino G, Grzeschik KH, Jaye M, et al: Regional localization on the human X chromosome and polymorphism of the coagulation factor IX gene (hemophilia B locus). Proc Natl Acad Sci U S A 81:498–502, 1984.
46. Hougie C, Barrow EM, Graham JB: Stuart clotting defect. I. Segregation of an hereditary hemorrhagic state from the heterogeneous group

heretofore called stable factor (SPCA, proconvertin, factor VII) deficiency. J Clin Invest 36:485–496, 1957.
47. Scambler PJ, Williamson R: The structural gene for human coagulation factor X is located on chromosome 13q34. Cytogenet Cell Genet 39:231–233, 1985.
48. Thompson RE, Mandle R, Kaplan AP: Association of factor XI and high molecular weight kininogen in human plasma. J Clin Invest 60:1376–1380, 1977.
49. Meijers JC, Tekelenburg WL, Bouma BN, et al: High levels of coagulation factor XI as a risk factor for venous thrombosis. N Engl J Med 342:696–701, 2000.
50. Thomas Renne´ ,Alvin H. Schmaier, Katrin F. Nickel, Margareta Blomba¨ and Coen Maas. In vivo roles of factor XII.From www.bloodjournal.org by guest on September 5, 2018.
51. Samuel M, Pixley RA, Villanueva MA, Colman RW, Villanueva GB. Human factor XII (Hageman factor) autoactivation by dextran sulfate: circular dichroism, fluorescence, and ultraviolet difference spectroscopic studies. J BiolChem. 1992;267(27):19691-19697.
52. Peyman Eshghi,Nader Cohan,Majid Naderi, Mehran Karimi. Factor XIII deficiency: a review of literature. 15-02-2010
53. Karimi M, Bereczky Z, Cohan N, Muszbek L. FactorXIII deficiency. Semin Thromb Hemost 2009; 35:426–438
54. Pike GN, Bolton-Maggs PHB. Factor Deficiencies in Pregnancy. Hematol Oncol Clin N Am 2011;25: 359–378
55. Timothy A. Springer von Willebrand factor, Jedi knight of the bloodstream MAJune 13, 2014; DOI 10.1182/blood-2014-05
56. Peter J. Lenting Olivier D. Christophe, Cécile V. Denis .Von Willebrand factor biosynthesis, secretion & clearance: connecting the far ends February 23, 2015; DOI 10.1182/blood-2014-06-528406
57. Furlan M, Robles R, Lammle B: Partial purification and characterization of a protease from human plasma cleaving von Willebrand factor to fragments produced by in vivoproteolysis. Blood 87:4223–4234, 1996.
58. Flood VH, Schlauderaff AC, Haberichter SL, et al. Crucial role for the VWF A1 domain in binding to type IV collagen. Blood. 2015;125(14):2297-2304.
59. La´szlo´ Muszbek,Zsuzsanna Bereczky,Bettina Kova´cs and Istva´n Koma´. Antithrombin deficiency and its laboratory diagnosis. DOI 10.1515/CCLM.2010.368
60. John H Griffin, Berislav V Zlokovic, andLaurent O Mosnier. Protein C anticoagulant and cytoprotective pathways. Published in final edited form as:Int J Hematol. 2012 April ; 95(4): 333–345. doi:10.1007/s12185-012-1059-0.

61. Chalmers E, Cooper P, Forman K, Grimley C, Khair K, Minford A, et al. Purpura fulminans:recognition, diagnosis and management. Arch.Dis.Child. 2011; 96:1066–1071. [PubMed: 21233082]
62. Mahasandana C, Suvatte V, Marlar RA, Manco-Johnson MJ, Jacobson LJ, Hathaway WE.Neonatal purpura fulminans associated with homozygous protein S deficiency. Lancet. 1990;335:61–62. [PubMed: 1967377]
63. Foley JH, Kim PY, Mutch NJ, Gils A. Insights into thrombin activatable fibrinolysis inhibitor function and regulation. J Thromb Haemost 11 Suppl 1: 306-315, 2013.
64. Yavuz Beyazit , Abdurrahim Sayilir , Alpaslan Tanoglu , Murat Kekilli , Erdem Kocak ,Fuat Ekiz and Adnan Tas Plasma Thrombin-activatable Fibrinolysis Inhibitor Levels Correlate with the Disease Activity of Ulcerative Colitis Article in Internal Medicine · July 2016
65. Bree Zimmerman and Leonard A. Valentino. Hemophilia: In Review. Pediatrics in Review 2013;34;289DOI: 10.1542/pir.34-7-289
66. Anna Mazurkiewicz-Pisarek, Grażyna Płucienniczak, Tomasz Ciach and Andrzej Płucienniczak. The factor VIII protein and its function Vol. 63, No 1/2016 .11–16
67. Vehar GA, Keyt B, Eaton D, Rodriguez H, O'Brien DP, Rotblat F, Oppermann H, Keck R, Wood WI, Harkins RN, Tuddenham EGD, Lawn RM and Capon DJ (1984) Structure of human factor VIII. Nature 312: 337–342.
68. Sixma JJ, van den Berg A. The haemostatic plug in haemophilia A: a morphological study of haemostatic plug formation in bleeding time skin wounds of patients with severe haemophilia A. Br J Haematol 1984;58:741–753. [PubMed: 6518139]
69. David Lillicrap .von Willebrand disease: advances in pathogenetic understanding, diagnosis, and therapy. First Edition paper, September 24, 2013; DOI 10.1182/blood-2013-06-498303
70. Englert JM, Moen JL, Wolberg AS. Altered fibrinopeptide release produces thicker fibrin fibers and an abnormal fibrin clot structure in an in vitro model of hemophilia B. International Fibrinogen Workshop. 2004
71. Lisman T, Mosnier LO, Thierry Lambert T, et al. Inhibition of fibrinolysis by recombinant factor VIIa in plasma from patients with severe hemophilia A. Blood 2002;99:175–179. [PubMed: 11756168]
72. Hong F, Leming W, Hongbao W (2007) The protein structure and effect of factor VIII. Thrombosis Res 119: 1–13.
73. Kasper CK, Lin JC. Prevalence of sporadic and familial haemophilia. Haemophilia 2007 Jan; 13(1): 90-2.
74. Mansouritorghabeh H, Rezaieyazdi Z, Pourfathollah AA. Haemophilia. Iran: Mashhad University of Medical Sciences;2005. P. 2-48.

75. Karin Knobe, Erik Berntorp Haemophilia and joint disease: pathophysiology, evaluation, and management, Sweden Journal of Comorbidity 2011;1:51–59
76. Soucie JM, Cianfrini C, Janco RL, Kulkarni R, Hambleton J, Evatt B, et al. Joint range-of-motion limitations among young males with hemophilia: prevalence and risk factors. Blood 2004;103:2467–73.
77. Alok Srivatasva ,World Federation of Hemophilia. Guidelines for the management of hemophilia. Montreal: World Federation of Hemophilia; 2005. Available from: http://www.wfh.org/2/docs/Publications/Diagnosis_ and_Treatment/Guidelines_Mng_Hemophilia.pdfNov 9, 2011].
78. Stephensen D, Tait RC, Brodie N, Collins P, Cheal R, Keeling D, et al. Changing patterns of bleeding in patients with severe haemophilia Haemophilia 2009;15:1210–14.
79. Silva M, Luck JV, Llinás A. Chronic haemophilic synovitis: the role of radiosynovectomy. In: Schulman S, editor. Treatment of Hemophilia Monographs No. 33, April 2004. Montreal: World Federation of Hemophilia; 2004. Available from: http://www.wfh.org/2/docs/Publications/Musculoskeletal_Physiotherapy/ TOH-33_English_ Synovectomy.pdf [Last accessed Dec 2, 2011].
80. Hakobyan N, Kazarian T, Jabbar AA, Jabbar KJ, Valentino LA. Pathobiology of haemophilic synovitis I: overexpression of mdm2. Blood 2004;104:2060–4.
81. Kasper CK. Hereditary plasma clotting factor disorders and their management. In: Schulman S, editor. Treatment of Hemophilia Monographs No. 4, April 2008. Montreal: World Federation of Hemophilia; 2008. Available from: http://www.wfh.org/2/docs/ Publications/Diagnosis_and_Treatment/TOH-4-Hereditary-Plasma-Revised2008.pdf [Last accessed Dec 2, 2011].
82. Zahida Qasim,* Nadeem Ikram,Khalid Hassan¨ Pattern of Haemarthrosis in Haemophilia. Journal of Rawalpindi Medical College (JRMC); 2013;17(2):204-206
83. Jamie O'Hara, Shaun Walsh, Charlotte Camp* , Giuseppe Mazza, Liz Carroll, Christina Hoxer and Lars Wilkinson O'Hara et al. The relationship between target joints and direct resource use in severe haemophilia Health Economics Review (2018) 8:1DOI 10.1186/s13561-018-0185-7
84. Sébastien Lobet,Cedric Hermans,Catherine Lambert Optimal management of hemophilic arthropathy and hematomas Journal of Blood Medicine 2014:5 207–218
85. Pettersson H, Ahlberg A, Nilsson IM. A radiologic classification of hemophilic arthropathy. Clin Orthop Relat Res. 1980;(149):153–159.

86. I. E. Morsing , P. Brons , J. M. Th. Draaisma , E. J. van Lindert , C. E. Erasmus Hemophilia A and Spinal Epidural Hematoma inChildren 28 October 2015.
87. Adriana Aparecida ,Ferreira Maria Teresa, Clinical and functional evaluation of the joint status of hemophiliac adults at a Brazilian blood center, Brazil DOI: 10.5581/1516-8484.20130010
88. Kar A, Mirkazemi R, Singh P, Potnis-Lele M, Lohade S, Lalwani A, Saha AS;Disability in Indian patients with haemophilia. Haemophilia. 2007;13(4):398-404.
89. Rizza CR, Spooner RJ, Giangrande Pl, UKHaemophilia Centre Doctors' Organization (UKCDO). Treatment of haemophilia in theUnited Kingdom 1981-1996. Haemophilia.2001;7:349-59. doi: 10.1111/j.1365-2516.2001.00521.x. PubMed PMID:11442638.
90. Hassan Mansouritorghabeh, MSc ,Clinical and Laboratory Approaches to Hemophilia A IJMSVol 40, No 3, May 2015
91. Ghosh K, Nair Ap, Jijina F, Madkaikar M, Shetty S, Mohanty D. Intracranial haemorrhage in severe haemophilia: prevalence and outcome in a developingcountry. Haemophilia. 2005;11:459-62. doi:10.1111/j.1365-2516.2005.01134.x. PubMedPMID: 16128888.
92. Benedik-Dolnicar M, Benedik M. Haematuriain patients with haemophilia and its influence on renal function and proteinuria.Haemophilia. 2007;13:489-92. doi:10.1111/j.1365-2516.2007.01445.x. PubMed PMID: 17880434.
93. Heiland M, Weber M, Schmelzle R. Lifethreatening bleeding after dental extraction in a hemophilia A patient with inhibitors to factor VIII: a case report. J Oral MaxillofacSurg. 2003;61:1350-3. doi: 10.1016/S0278-s2391(03)00739-0. PubMed PMID: 14613094.
94. Srivastava A, Brewer AK, Mauser-Bunschoten EP, Key NS, 56. Kitchen S, Llinas A, et al; Treatment Guidelines Working group on Behalf of the World Federation of Hemophilia. Guidelines for the management of hemophilia. Haemophilia 2013; 19 : e1-47.
95. Antonio Coppola Mirko Di Capua Treatment of hemophilia: a review of current advances and ongoing issues Journal of Blood Medicine 2010:1 183–195
96. Zahida Qasim,Nadeem Ikram, Khalid Hassan, Pattern of Haemarthrosis in Haemophilia Journal of Rawalpindi Medical College (JRMC); 2013;17(2):204-206
97. Shamsi TS and Chughtai AS. Haematological treatment options for haemophilia patients in countries with limited resources. In: Heijnen L, Sohail MT, eds. Comprehensive Haemophilia Care in Developing Countries. Lahore, Pakistan: Feroze sons; 2001; 26-33.
98. Radovan Kubeš, Peter Salaj, Rastislav Hromádka , Josef Včelák, Aleš Antonín Kuběna, Monika Frydrychová, Štěpán Magerský, Michal Burian,

Martin Ošťádal1 and Jan Vaculik .Range of motion after total knee arthroplasty in hemophilic arthropathy BMC Musculoskeletal Disorders (2018) 19:162

99. M.Elaine eyster et.al.,The bleeding time is longer than normal in haemophilia. blood vol.58.no.4.,oct 1981

100. Esther R. van Bladel, Mark Roest, Philip G. de Groot, and Roger E.G. Schutgens Up-regulation of platelet activation in hemophilia A doi:10.3324/haematol.2011.042663

101. Adcock DM, Kressin DC, Marlar RA: Effect of 3.2% vs 3.8% sodium citrate concentration on routine coagulation testing, Am J Clin Pathol 107:105–110, 1997.

102. Lippi G, Meschi T, Borghi L. Variation of activated partial thromboplastin time according to age and sex in a large population study: analytical and clinical implications.Blood Coagul Fibrinolysis. 2012;23:177-8. doi:10.1097/MBC.0b013e32834ee0fb. PubMed PMID: 22270798.

103. Wayne, PA, NCCLS: Collection, transport, and processing specimens for coagulation testing and performance of coagulation assay. 4th ed.Approved guideline. NCCLS Document H21-A4. 2003. p. 1-24. Available from: http://shop.clsi.org/site/Sample_PDFs/H21A5_sample.pdf.

104. Karen A. Moser and Dorothy M. (Adcock) Funk*Am. J. Chromogenic factor VIII activity assay Hematol. 89:781–784, 2014. VC 2014 Wiley eriodicals, Inc.

105. Wayne L. Chandler, MD, Chris Ferrell, MT(ASCP), Joo Lee, MT(ASCP), Theingi Tun, MT(ASCP),and Hien Kha, MT(ASCP) Comparison of Three Methods for Measuring Factor VIIILevels in Plasma DOI: 10.1309/C8T8YNB4G3W45PRF

106. Chang SH, Tillema V, Scherr D: A percent correction formula for evaluation of mixing studies, Am J Clin Pathol 117:62–73, 2002.

107. Kitchen S, McCraw A, Echenaguicia M. Diagnosis of Hemophilia and Other Bleeding Disorders:A Laboratory Manual., 2nd ed. Montreal, Quebec, Canada: World Federation of Hemophilia;2010

108. Eich S, Kusch M, Grundmann C, et al. Factor VIII determination in patient's plasma and concentrates: A novel test equally suited for both matrices. Blood Coagul Fibrinolysis 2003;14:347–353.

109. Assay of factor VIII antigen (VIII:CAg) in 294 Haemophilia A patients by a new commercial ELISA using monoclonal antibodies Article *in* Haemophilia · March 1998

110. Kasper CK. Hereditary plasma clotting factor disorders and their management. In: Schulman S, editor. Treatment of HemophiliaMonographs No. 4, April 2008. Montreal: World Federation of Hemophilia; 2008. Available from:

http://www.wfh.org/2/docs/Publications/Diagnosis_and_Treatment/TOH-4-Hereditary-Plasma-Revised2008.pdf
111. S.B. Somwanshi, V.B. More, S.N. Hiremath, R.T. Dolas, K.B. Kotade, V.M.Gaware .Hemophilia-Inherited Bleeding Disorder: An Overview Volume 3, Issue 3, 596-620. Accepted On 07 March 2014
112. Denise Rodrigues Holsbach,Maria Lúcia Ivo, Alexandra Maria AlmeidaCarvalho,Albert Schiaveto de Souza Quality of Life and Viral Infections in Adults with Hemophilia. International Medical Society http://imedicalsociety.org12016Vol. 9 No. 347doi: 10.3823/2218
113. Bonn, Optimal treatment strategies for hemophilia: achievements and limitations of current prophylactic regimens Johannes Oldenburg Institute of Experimental Haematology and Transfusion Medicine, University Clinic, GermanyBlood First Edition paper, February 23, 2015; DOI 10.1182/blood- 2015-01-528414.
114. Jan astermark, Lilian tengborn, Primary prophylaxis in severe haemophilia should be started at an early age but can be individualized.DOI:1999
115. Nidra I. Rodriguez, MD, W. Keith Hoots, MDAdvances in Hemophilia: Experimental Aspects and Therapydoi:10.1016/j.pcl.2008.01.010
116. Jerry S Powell ,Recombinant factor VIII in the management of hemophilia A: current use and future promise Therapeutics and Clinical Risk Management 2009:5 391–402
117. Fijnvandraat K, Peters M, ten Cate JW. Inter-individual variation in half-life of infused recombinant factor VIII is related to pre-infusion von Willebrand factor antigen levels. Br J Haematol. 1995;91:474-476.
118. Molho P, Rolland N, Lebrun T, et al. Epidemiological survey of the orthopaedic status of severe haemophilia A and B patients in France. The French Study Group. secretariat.haemophiles@cch.ap-hop-paris.fr. Haemophilia. 2000;6:23-32.
119. Villar A, Jimenez-Yuste V, Quintana M, et al. The use of haemostatic drugs in haemophilia:desmopressin and antifibrinolytic agents. Haemophilia 2002;8:189–93.
120. Leissinger C, Becton D, Cornell C Jr, Cox Gill J.High-dose DDAVP intranasal spray (Stimate) forthe prevention and treatment of bleeding in patientswith mild haemophilia A, mild or moderate type 1von Willebrand disease and symptomatic carriers ofhaemophilia A. Haemophilia 2001;7(3):258-66.
121. Mannucci PM. Hemostatic drugs. N Engl J Med 1998 Jul23;339(4):245-53.
122. Thierry Burnouf Local Hemostatic BloodProducts In Hemophilia Care: Fibrin Sealant And Platelet Gel Second Edition Miryana Radosevich Human Plasma Product Services (Hpps) April 2008 • No 36

123. Stanworth SJ. The evidence-based use of FFP and cryoprecipitate for abnormalities of coagulation testsand clinical coagulopathy. Hematology Am Soc Hematol Educ Program 2007:179-86.
124. Chuansumrit A, Isarangkura P, Chantanakajornfung A, et al. The efficacy and safety of lyophilized cryoprecipitate in hemophilia A. J Med Assoc Thai 1999;82(Suppl 1):S69-73.
125. Samuel L. Murphy and Katherine A. Gene therapy for haemophilia.British Journal of Haematology, 140, 479–487 doi:10.1111/j.1365-2141.2007
126. Eckhardt, C.L. Inhibitor development in nonsevere hemophilia A (2014).
127. Guh S, Grosse SD, McAlister S, Kessler CM, Soucie JM. Healthcare expenditures for males with haemophilia and employer-sponsored insurance in the United States, 2008. Haemophilia 2012; 18:268-75.
128. Lollar P: Pathogenic antibodies to coagulation factors: I. Factor VIII and factor IX. J Thromb Haemost 2:1082, 2004.
129. Gouw, S.C.; van den Berg, H.M.; Fischer, K.; Auerswald, G.; Carcao, M.; Chalmers, E.; Chambost, H.;Kurnik, K.; Liesner, R.; Petrini, P.; et al. Intensity of factor VIII treatment and inhibitor development in children with severe hemophilia A: The RODIN study. Blood **2013**, 121, 4046–4055.
130. Marijke van den Berg .Risk of Inhibitor Development in Children with Haemophilia A DOI: 10.17925/EOH.2007.0.0.8
131. Astermark J, Altisent C, Batorova A, et al; European Haemophilia Therapy Standardisation Board. Non-genetic risk factors and the Development of inhibitors in haemophilia: a comprehensive review and consensus report. Haemophilia 2010;16(5):747-66. Dr. Gwen
132. R.Schwaab,H.h.Brackmann,C.Meyer.Haemophilia A: Mutation Type Determines Risk of Inhibitor Formation.Article in Thrombosis and Haemostasis · January 1996
133. Schwaab R, Brackmann HH, Meyer C et al. Haemophilia A: mutation type determines risk of inhibitor formation. Thromb Haemost 1995; 74: 1402–6.
134. A Samantha Claudia ,The etiology of inhibitor development in children with severe hemophilia Gouw geboren op 2 juli 1978 te RotterdamUtrecht, 2011
135. Roberts HR: Inhibitors and their management, in Hemophilia & Other Bleeding Disorders, edited by Rizza, G Lowe, p 371. WB Saunders, New York, 1997.
136. Kasper CK, Aledort L, Aronson D, et al. Proceedings: A more uniform measurement of factor VIII inhibitors. Thromb Diath Haemorrh. 1975;34(2):612.

137. Verbruggen B, Novakova I, Wessels H, Boezeman J, van den BM, Mauser- Bunschoten E. The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. Thromb Haemost. 1995;73(2):247–251.
138. Biggs R, Bidwell E. A method for the study of antihaemophilic globulin inhibitors with reference to six cases. Br J Haematol. 1959;5:379–395.
139. M van Geffen, M Dardikh and B Verbruggen, Factor VIII Inhibitor Assays: Methodology, Shortcomings,and Challenges.Affiliation: Laboratory of Hematology, Department of Laboratory Medicine, Radboud University Nijmegen Medical Center, Nijmegen, The NetherlandsJCD 2009; 000:(000). Month 2009 1 www.slm-hematology.com
140. Giles AR, Verbruggen B, Rivard GE, Teitel J, Walker I. A detailed comparison of the performance of the standard versus the Nijmegen modification of the Bethesda assay in detecting factor VIII:C inhibitors in the haemophilia A population of Canada. Association of Hemophilia Centre Directors of Canada. Factor VIII/IX Subcommittee of Scientific and Standardization Committee of International Society on Thrombosis and Haemostasis. Thromb Haemost. 1998;79(4):872–875.
141. Angiola Rocino , Massimo Franchini and Antonio Coppola .Treatment and Prevention of Bleeds in Haemophilia Patients with Inhibitors to Factor VIII/IX J. Clin. Med. 2017, 6, 46; doi:10.3390/jcm6040046
142. Valentino, L.A.; Kempton, C.L.; Kruse-Jarres, R.; Mathew, P.; Meeks, S.L.; Reiss, U.M.; International Immune Tolerance Induction Study Investigators. US Guidelines for immune tolerance induction in patients with haemophilia A and inhibitors. Haemophilia 2015, 21, 559–567.
143. Saulius Butenas, Kathleen E. Brummel, Richard F. Branda, Sara G. Paradis, And Kenneth G.Mechanism Of Factor Viia–Dependent Coagulation In Hemophilia Blood. 1 February 2002 _ Volume 99, Number 3
144. Geir E Tjønnfjord Factor eight inhibitor bypass activity (FEIBA) in the management of bleeds in hemophiliapatients with high-titer inhibitors 2007:3(4) 527–531
145. Sonata Saulyte Trakymiene Musculoskeletal Pathologies In Children With Haemophilia Evaluated Using A Standardised Physical Joint Scoring System To Assess Disability 2011.
146. Scharr, University Of Sheffield, Sheffield, S1 4DA, UK. J.P.Wight@Sheffield.Ac.Uk Citation Haemophilia. 2003 Jul;9(4):418-35
147. Agarwal et al. Classical haemophilia. J Assoc Physicians India. 1981;29:120-130.

148. T. B. Sadaria, H. M. Goswami, Safal Patel Study Of Laboratory Parameters In Hemophilia Patients Int J Cur Res Rev | Vol 8 • Issue 17 • September 2016
149. Stevan MM. Madhok YR, Forbes CD, Sturrock RD. Haemophilic arthritis. Quat J of Med 1986;58:181- 197.
150. Kitchens CS. Occult haemophilia. Johns Hopkins Medical J. 1980;746:255-259.
151. Takim & Shrivastava. A current situation of regular replacement therapy for haemophilia in Japan. Hamophila 2009.15.78-82.
152. Tlacuilo-Parra A, Villela-Rodriguez J, Garibaldi-Covarrubias R, Soto-Padilla J, Orozco-Alcala J.Functional independence score in hemophilia: A cross-sectional study assessment of Mexican children. Pediatric blood & cancer.2010;54(3):394-7..
153. Just An Unfortunate Coincidence ‘:Children’s Understanding Of Haemophilia Genetics & Inheritance. K.Khair, F.Gibson, L.Meerabeau . Haemophilia (2011) ,17, 470-475
154. Anita Kar, Supriya Phadnis, Sumedha Dharmarajan & Juhi Nakade Epidemiology & social costs of haemophilia in India Indian J Med Res 140, July 2014, pp 19-31
155. Ajay Hegde, Rajesh Nair, Sunil Upadhyaya. Spontaneous intracerebral hemorrhage in hemophiliacs—A treatment dilemma doi:oct 25,2016.
156. Revue Du Rhumatisme; Iliopsoas Hematoma In Patients With Hemophilia: A Single-Center Study Marc Dauty Joint, Bone, Spine: 74(2): In Article179-83 · April 2007 With 115 Reads DOI: 10.1016/J.Jbspin.2006.05.014 · Source: Pubmed
157. Sagir G. Ahmed et.al.,The frequency of iron deficiency among patients with haemophilia-A in northern Nigeria: correlation with the disease severity and clinical implications DOI: 10.4103/1110-1067.161294 Egyptian Journal of Haematology 2015, Vol 40 No 2
158. Paranthaman Poongavanam et.al., The Frequency of Iron Deficiency among Patients with Hemophilia DOI: 10.9790/0853-1606080409 June. 2017
159. Sam Schulman , Mild Hemophilia Treatment Of Hemophilia November 2012 No. 41

PRFORMA

Name: **Age/Sex:** **IP N0:**

Presenting complaints:

Personal history:

Age of diagnosis of disease-

Family history:

Any relatives suffering from the disease - yes no

If so, how many-

Treatment history:

Time of first administration of factor VIII-

Time interval between factor VIII administration-

Other treatments undertaken-

Physical examination:

	Yes	No
1. Swelling		
2. Muscle atrophy		
3. Crepitus		
4.Joint deformities		
5.Gait change		

Disability Assessment

Self Care:	Score 1	Score2	Score3	Score4
1.Grooming eating				
2.Bathing				
3.Dressing				
Transfers				
4.Chair Transfer				

5. Squatting

Locomotion

6.Walking

7.Step climbing

LABORATORY PARAMETERS:

CBC

Coagulation profile

PT

APTT

INR

TREATMENT GIVEN:

நோயாளிகளுக்கு அறிவிப்பு மற்றும் ஒப்புதல் படிவம்
(மருத்துவ ஆய்வில் பங்கேற்பதற்கு)

ஆய்வு செய்யப்படும் தலைப்பு:
பங்கு பெறுவரின் பெயர்:
பங்கு பெறுவரின் வயது:

		பங்கு பெறுவர் இதனை ✓ குறிக்கவும்
1.	நான் மேலே குறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்களை படித்து புரிந்து கொண்டேன். என்னுடைய சந்தேகங்களை கேட்கவும், அதற்கான தகுந்த விளக்கங்களை பெறவும் வாய்ப்பளிக்கப்பட்டுள்ளது என அறிந்து கொண்டேன்.	☐
2.	நான் இவ்வாய்வில் தன்னிச்சையாக தான் பங்கேற்கிறேன். எந்த காரணத்தினாலோ எந்த கட்டத்திலும், எந்த சட்ட சிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகி கொள்ளலாம் என்றும் அறிந்து கொண்டேன்.	☐
3.	இந்த ஆய்வு சம்பந்தமாகவோ, இதை சார்ந்து மேலும் ஆய்வு மேற்காள்ளும் போதும் இந்த ஆய்வில் பங்குபெறும் மருத்துவர் என்னுடைய மருத்துவ அறிக்கைகளை பார்ப்பதற்கு என் அனுமதி தேவையில்லை என அறிந்து கொள்கிறேன். நான் ஆய்வில் இருந்து விலகிக் கொண்டாலும் இது பொருந்தும் என அறிகிறேன்.	☐
4.	இந்த ஆய்வின் மூலம் கிடைக்கும் தகவலையோ, முடிவையோ பயன்படுத்திக் கொள்ள மறுக்க மாட்டேன்.	☐
5.	இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக் கொள்கிறேன் எனக்கு கொடுக்கப்பட்டஅறிவுரைகளின் படி நடந்து கொள்வதுடன், ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்று உறுதியளிக்கிறேன். என் உடல் நலம் பாதிக்கப்பட்டாலோ, அல்லது எதிர்பாராத, வழக்கத்திற்கு மாறான நோய்குறி தென்பட்டாலோ உடனே இதை மருத்துவ அணியிடம் தெரிவிப்பேன் என உறுதி அளிக்றேன்.	☐

பங்கேற்பவரின் கையொப்பம் / ..... இடம் .....
கட்டைவிரல் ரேகை
பங்கேற்பவரின் பெயர் மற்றும் விலாசம் .....
ஆய்வாளரின் கையொப்பம் /..... இடம் .....
ஆய்வாளரின் பெயர் .....
மையம் .....
கல்வியறிவு இல்லாதவற்கு (கைரேகை வைத்தவர்களுக்கு) இது அவசியம் தேவை
சாட்சியின் கையொப்பம் /..... இடம் .....
பெயர் மற்றும் விலாசம் ....

S. No.	IP.No.	Age	Sex	Disease - Hemophilia A/B	Inhibitor Status	classification of the disease	PT (Sec)	APTT (Sec)	FISH Score	Platelet Count	Hb gm/dl	Family History	h/o Blood Tranfusion	Treatment Given	Joints affected	Soft tissue bleeding	Total No. of Admission	CNS & other life threatening haemorrhage
1	55652	35	M	Hemophilia A	Negative	severe	16	76.8	19	43000	13	Yes	Yes	Inj. F-VIII	Left Knee	Yes	14	No
2	57253	25	M	Hemophilia A	Negative	severe	10.4	48.8	19	3.33 Lakhs	12.3	No	Yes	Inj. F-VIII	Right Knee	Yes	18	Yes
3	58552	30	M	Hemophilia A	Negative	severe	15	40.8	18	3.85 Lakhs	12.5	Yes	Yes	Inj. F-VIII	Left Knee	No	9	No
4	58551	14	M	Hemophilia A	Negative	severe	15.4	45.3	18	3.85 Lakhs	11.5	No	No	Inj. F-VIII	Left Knee	Yes	10	No
5	59207	20	M	Hemophilia A	Negative	severe	14.3	39.8	21	2.25 Lakhs	13.5	Yes	Yes	Inj. F-VIII	Right Knee	No	6	No
6	59884	25	M	Hemophilia A	Negative	severe	14.5	42.8	19	2.75 Lakhs	11.8	Yes	Yes	Inj. F-VIII	Both Knee	Yes	22	No
7	58110	20	M	Hemophilia A	Positive	severe	14.1	92.7	20	8.03 Lakhs	17.4	No	No	Inj. Nova Seven	Right Knee	No	8	Yes
8	60090	19	M	Hemophilia B	Negative	severe	14.8	42.8	20	4.25 Lakhs	14.5	No	No	Inj.F IX	Left Knee	Yes	4	No
9	62867	22	M	Hemophilia A	Negative	severe	9.3	46.4	24	1.75 Lakhs	13.6	No	No	Inj. F-VIII	Both Elbow joints	Yes	26	No
10	63957	13	M	Hemophilia A	Negative	severe	11.4	36.5	25	1.05 Lakhs	12.8	Yes	No	Inj. F-VIII	Left knee	No	9	No
11	63786	26	M	Hemophilia A	Negative	severe	11.8	39.8	28	3.35 Lakhs	14	Yes	No	Inj. F-VIII	Both Ankle joints	Yes	52	No
12	66633	32	M	Hemophilia A	Negative	severe	13	52.5	19	2.98 Lakhs	9	No	Yes	Inj. F-VIII	Both Knee joints right elbow	No	18	Yes
13	67934	40	M	Hemophilia A	Negative	severe	14.1	39.4	20	2.90 Lakhs	9.6	No	Yes	Inj. F-VIII	Left Knee Left hip joint	No	11	No
14	67935	20	M	Hemophilia A	Negative	Mild	9.9	39.2	28	2.11 Lakhs	13.3	Yes	No	Inj. F-VIII	-	Yes	15	No
15	70431	20	M	Hemophilia A	Negative	severe	13.7	52.4	23	1.15 Lakhs	11.4	Yes	Yes	Inj. F-VIII	Right shoulder	Yes	14	No
16	23859	15	M	Hemophilia B	Negative	severe	12.8	48.2	22	3.82 Lakhs	14.2	Yes	Yes	Inj. F-IX	Right Knee	No	18	No
17	21542	36	M	Hemophilia A	Negative	severe	11.8	49.3	22	2.95 Lakhs	10.8	Yes	Yes	Inj. F-VIII	Both Knee	No	9	No
18	15733	14	M	Hemophilia B	Negative	severe	14.5	38.8	21	2.88 Lakhs	12.5	Yes	No	Inj. F IX	Left Knee Left ankle joint	No	11	No
19	21321	24	M	Hemophilia A	Negative	severe	12.1	44.1	28	5.04 Lakhs	14.1	Yes	Yes	Inj. F-VIII	-	Yes	12	No

S. No.	IP.No.	Age	Sex	Disease - Hemophilia A/B	Inhibitor Status	classification of the disease	PT (Sec)	APTT (Sec)	FISH Score	Platelet Count	Hb gm/dl	Family History	h/o Blood Tranfusion	Treatment Given	Joints affected	Soft tissue bleeding	Total No. of Admission	CNS & other life threatening haemorrhage
20	1699	23	M	Hemophilia A	Negative	severe	11.2	59.2	21	3.90 Lakhs	13.6	Yes	Yes	Inj. F-VIII	Left Knee jint	No	15	No
21	26313	54	M	Hemophilia A	Negative	severe	12.3	38.4	22	3.66 Lakhs	12.1	Yes	Yes	Inj. F-VIII	Right Knee joint	No	8	No
22	15691	18	M	Hemophilia A	Negative	severe	14.2	39.8	22	2.98 Lakhs	11.6	Yes	No	Inj. F-VIII	Left hip & Left Knee	Yes	12	No
23	27363	37	M	Hemophilia A	Negative	severe	15.8	45.1	19	1.23 Lakhs	12.8	Yes	Yes	Inj. F-VIII	Right Knee joint	No	7	No
24	16435	70	M	Hemophilia A	Negative	severe	13.8	108.2	15	3.32 Lakhs	13.2	Yes	Yes	Inj. F-VIII	Left Knee	No	4	No
25	18466	20	M	Hemophilia A	Positive	severe	13	42.01	25	2.98 Lakhs	12.6	Yes	No	Inj. Novaseven	Right ankle	No	12	No
26	19575	13	M	Hemophilia A	Negative	Mild	14.8	40.3	28	3.34 Lakhs	14.8	No	No	Inj. FVIII	-	Yes	7	No
27	19620	53	M	Hemophilia A	Negative	severe	13.2	80.6	18	2.32 Lakhs	12.3	No	Yes	Inj. FVIII	Right Knee & Right hip	No	11	No
28	20216	20	M	Hemophilia A	Positive	severe	13.8	39.5	25	2.35 Lakhs	11.8	Yes	Yes	Inj. Nova seven	Left Knee	No	6	No
29	32415	15	M	Hemophilia A	Positive	severe	15.6	40.2	20	2.92 Lakhs	13.6	No	No	Inj. Nova seven	Left Knee	No	7	No
30	21569	14	M	Hemophilia A	Negative	severe	13.6	39.4	24	3.36 Lakhs	12.2	Yes	No	Inj. FVIII	Right Elbow	No	7	No
31	22089	18	M	Hemophilia A	Negative	severe	14.6	42.8	24	2.92 Lakhs	9.8	No	Yes	Inj. FVIII	Right ankle	Yes	6	No
32	24916	18	M	Hemophilia B	Negative	severe	13.8	39.8	23	3.34 Lakhs	11.8	No	No	Inj. FIX	Left Knee	No	4	No
33	24945	13	M	Hemophilia A	Negative	severe	13.6	41	20	3.84 Lakhs	12	Yes	No	Inj. FVIII	-	Yes	7	No
34	29143	30	M	Hemophilia A	Negative	severe	12.8	102.2	17	4.1 Lakhs	13.9	Yes	No	Inj. FVIII	Left ankle	Yes	22	No
35	90982	24	M	Hemophila B	Negative	severe	13	51.8	24	4.25 lakhs	12.8	No	Yes	Inj. FIX	Left Knee	No	6	No